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EFFECT OF NITRATE APPLICATIONS UPON THE HYDROCYANIC-ACID CONTENT OF SORGHUM[1]

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INTRODUCTION

It is now generally recognized that growing plants of the sorghum family contain hydrocyanic acid, which is present largely as a glucoside (dhurrin), from which hydrocyanic acid is set free by the action of an enzym normally present in the sorghum plant. Under conditions favorable to the action of this enzym all the hydrocyanic acid is liberated, the time required varying from less than 2 hours at 45° C., to less than 20 hours at room temperature (20° C.).

The proportion of hydrocyanic acid varies much, young plants generally containing a higher percentage than more mature ones grown under like conditions. In green plants the leaves contain a higher percentage than the stems, a fact which may explain part of the difference found between young and old plants, as the leaves of the former constitute a much greater proportion of the total weight of the plant.

In 1903 Brünnich,[2] in Australia, found that sorghum plants fertilized with sodium nitrate contained more hydrocyanic acid than those grown on unfertilized soil.

Four years later Alway and Trumbull,[3] in Nebraska, from the analysis of large, dark green, and of small, yellowish green sorghum plants growing as volunteers, concluded that the higher percentage of prussic acid found in the larger and greener plants was due to more available nitrogen in the soil where they grew.

In 1915 Willaman and West[4] concluded from field experiments in Minnesota that "on soils deficient in nitrogen, added nitrogen may slightly increase the prussic acid in sorghum," while "with a fertile soil and abundant nitrogen this effect may not be produced",[5] and from later studies[6] that, "unhealthy plants usually contain more hydrocyanic acid than healthy ones. The unhealthy condition may be due to malnutrition, to improper transpiration, to insect pests, or to other causes," while "adequate water supply is usually accompanied by low, and inadequate by high, hydrocyanic-acid content." Vinall[7] from a critical

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[2] BRÜNNICH, J. C. HYDROCYANIC ACID IN FODDER PLANTS. *In* Jour. Chem. Soc. [London], v. 83, pt. 2, p. 788–796. 1903.

[3] ALWAY, F. J., and TRUMBULL, R. S. ON THE OCCURRENCE OF PRUSSIC ACID IN SORGHUM AND MAIZE. *In* Nebr. Agr. Exp. Sta. 23d Ann. Rpt., 1909, p. 35–36. 1910.

[4] WILLAMAN, J. J., and WEST, R. M. NOTES ON THE HYDROCYANIC-ACID CONTENT OF SORGHUM. *In* Jour. Agr. Research, v. 4, p. 179–185. 1915.

[5] ——— ——— OP. CIT., p. 184.

[6] ——— ——— THE EFFECT OF CLIMATIC FACTORS ON THE HYDROCYANIC-ACID CONTENT OF SORGHUM. *In* Jour. Agr. Research, v. 6, p. 271–272. 1916.

[7] VINALL, H. N. A STUDY OF THE LITERATURE CONCERNING POISONING OF CATTLE BY THE PRUSSIC ACID IN SORGHUM, SUDAN GRASS, AND JOHNSON GRASS. *In* Jour. Amer. Soc. Agron., v. 13, p. 267–280. 1921.

survey of the literature concludes that injury to growing sorghum plants by frost or drought increases the prussic-acid content, but that stunted growth from lack of plant food in the soil diminishes it.

In view of these divergent conclusions as to the effect of available nitrogen upon the hydrocyanic-acid content, and hence as to the possibility of using sorghum as an indicator in studies of the availability of nitrogen, the writer decided to try the effect of varying amounts of nitrate upon plants otherwise subjected to the same conditions.

EXPERIMENTAL DATA

SOILS USED

The plants analyzed were grown in the greenhouse in stoneware jars 8.3 inches in diameter and 8.5 inches deep. Each of the three Minnesota soils selected for use in the experiment were treated alike except in the amount of fertilizers applied. The soils were all low in nitrogen, so that it was to be expected that the addition of nitrogen would produce a marked visible effect on the plants. They were:

Thurston loam (subsoil), from the farm of the Minnesota Agricultural Experiment Station (nitrogen 0.039 per cent).

Merrimac loamy sand, from Coon Creek Sand Experimental Fields (nitrogen 0.068 per cent).

Coloma sand, from Nickerson, in Carlton County (nitrogen 0.068 per cent).

TREATMENT OF SOILS

Before the Thurston loam was placed in the jars, muriate of potash (52 per cent K_2O) and superphosphate (45 per cent P_2O_5) were mixed into the soil, each at the rate of 2 pounds per ton (0.1 per cent).

This was equivalent to an application of 1 ton per acre of each of the fertilizers, an amount that should remove any question of a lack of either potash or phosphate. Half the jars received no nitrate, while of the other half each jar received 0.25 gm. at each application, this being given in solution at recorded intervals. The plants were very pale in color and made very little growth where liberal amounts of only potash and phosphoric fertilizers were added, but where sodium nitrate in addition was applied the color was dark green and the growth was excellent.

The Merrimac loamy sand is low in nitrogen, but it had just previously been used in the greenhouse for growing alfalfa, and its available nitrogen had thereby been increased. As this soil had been found not to respond to either potash or phosphate with farm crops, neither of these was added. With this soil, in the early stages of growth there was no difference in appearance between the plants that had received nitrate and those that had not, growth and color being good in both, but later, when some of the nitrogen furnished by the alfalfa roots had been used up, a difference appeared.

On the Coloma sand, to which neither potash nor phosphate was supplied, the growth of the sorghum was very poor even where nitrate was added.

SORGHUM FROM FIRST PLANTING

All the jars of the three soils were planted with seed from the same lot on December 5, 1922. The growth from this planting was slow, due to the short period of sunshine daily in the winter. The plants grown from

this seeding were all used in perfecting the manipulative procedure, and for these there are no quantitative data. Qualitative tests showed that those plants grown with added nitrate contained a higher proportion of hydrocyanic acid.

METHOD OF ANALYSIS

The method of analysis thus developed and used with the subsequent plantings was as follows:

The fresh green material was weighed, passed through a food chopper, and placed in a Kjeldahl flask, with not more than 500 cc. of water, using only enough to wash the sorghum out of the chopper into the flask. This mixture was allowed to autolyze for two hours at 45° C., or over night at room temperature, and then, without addition of acid, distilled into dilute sodium hydroxid solution. The basic distillate was then treated with 2 or 3 cc. of a 3 per cent solution of ferrous sulphate, and allowed to stand about 20 minutes, by which time part but not all of the iron was oxidized to the ferric state, and then made acid with dilute sulphuric acid. The blue color developed slowly or quickly, according to the amount of hydrocyanic acid present, and on this account it was found best for the test solution to stand for some hours before being compared in a Campbell-Hurley colorimeter with the standard color prepared from potassium cyanid.

It was found convenient to make the standard equivalent to 10 mgm. of potassium cyanid per 100 cc. of standard solution, and usually about 30 gm. of the green sorghum was used for each determination, but in the case of green leaves, or of very young whole plants, much smaller samples sufficed, while with large, stocky plants, larger samples were necessary. As about 150 cc. of distillate was usually secured, the volume of the test solution was made up to 200 cc. except in cases of samples that contained unusually large amounts of hydrocyanic acid, when the volume was increased.

SORGHUM FROM LATER PLANTINGS

As soon as the first crop had been harvested, seed was planted among the roots, on January 30, and the second crop grown without cultivation or addition of any fertilizer except nitrate.

The plants of the second crop on the Thurston loam subsoil, where no nitrate had been added, were short, slender, and very light in color, indicating extreme nitrogen hunger, and did not furnish enough hydrocyanic acid to permit of its quantitative determination, or in some cases even to permit of its detection (Table I). Even the lighter applications of nitrate produced marked improvement in growth and color, and gave readily measurable amounts of hydrocyanic acid. Larger applications of nitrate raised the hydrocyanic-acid content proportionately.

The nitrogen hunger was not marked in the first crop on the Merrimac loamy sand, and even in the second crop it was not extreme. Although the available nitrogen in the unfertilized jars had been diminished by the first crop, there still remained enough to give a growth to the plants of the second crop better than on either of the other soils. In the case of lots E and F, there was no difference in appearance between the plants grown with and without nitrate, but in lot I the plants without nitrate were much lighter in color. In every lot the percentage of hydrocyanic acid was considerably increased by the addition of nitrate (Table II).

TABLE I.—*Sorghum plants grown on Thurston loam subsoil, with nitrate applied at intervals*

Lot.	Jar number.	Age of plants.	Nitrate applied.		Average height.	Average weight.	Hydrocyanic acid (HCN).	
			Per jar.	Per acre.			Per cent.	Per plant.
		Days.	*Gm.*	*Lb.*	*Cm.*	*Gm.*		*Mgm.*
A	1	61	None.	None.	16	0.3	None.	None.
A	2	61	1.25	314	51	7.0	0.0082	0.573
A	3	61	1.25	314	40	5.0	.0070	.350
A	4	61	1.50	376	43	6.0	Trace.	Trace.
A	5	61	1.75	440	40	5.0	.0028	.140
A	6	61	3.25	815	53	9.0	.0049	.430
B	7	64	1.25	314	46	4.0	.0012	.048
B	8	64	1.25	314	.....	5.0	.0033	.165
B	9	64	2.00	502	61	10.0	.0098	.980
B	10	64	5.25	1,317	56	9.0	.0439	3.940
C	11	75	None.	None.	13	0.3	None.	None.
C	12	75	1.50	376	40	3.3	.0029	.096
C	13	75	1.50	376	.....	3.3	.0078	.258
C	14	75	1.75	440	33	4.5	.0016	.072
C	15	75	2.25	564	33	3.7	.0094	.330
C	16	75	10.25	2,572	33	4.5	.0082	.370
D	17	86	1.50	376	48	4.0	.0082	.330
D	18	86	1.75	440	56	4.0	.0348	1.390
D	19	86	2.00	502	46	3.0	.0340	1.050
D	20	86	2.50	625	53	4.0	.0353	1.410
D	21	86	15.25	3,827	53	5.5	.1313	7.250

TABLE II.—*Sorghum plants grown on Merrimac loamy sand with and without nitrate applications*

Lot.	Jar number.	Age of plants.	Nitrate applied.		Average height.	Average weight.	Hydrocyanic acid (HCN).		Part of plant analyzed.
			Per jar.	Per acre.			Per cent.	Per plant.	
		Days.	*Gm.*	*Lb.*	*Cm.*	*Gm.*		*Mgm.*	
E	22	46	None.	None.	.....	10.0	0.0123	1.230	Whole plant.
E	23	46	1.25	310	.....	9.0	.0246	2.214	Do.
F	24	48	None.	None.	.....	.....	.0098	.....	Leaves only.
F	25	48	1.25	310	.....	.....	.0836	.....	Do.
G	26	63	None.	None.	61	7.0	.0234	1.638	Whole plant.
G	27	63	1.50	375	71	13.0	.0314	4.080	Do.
H	28	72	None.	None.	46	3.5	None.	None.	Do.
H	29	72	None.	None.	.....	4.0	Trace.	Trace.	Do.
H	30	72	1.50	375	56	16.0	.0369	5.91	Do.
H	31	72	1.50	375	.....	10.0	.0345	3.45	Do.
I	32	89	None.	None.	56	7.0	.0041	.280	Do.
I	33	89	2.00	502	69	10.0	.1100	11.00	Do.
I	34	89	2.00	502	64	9.0	.0460	4.14	Do.

All the plants grown on the Coloma sand were small, but the difference both in size and percentage of hydrocyanic acid content was noticeably in favor of those treated with nitrate. The total amount of hydrocyanic acid per plant was a hundred times as great in the latter (Table III).

TABLE III.—*Sorghum grown on Coloma sand, with and without nitrate application*

Lot.	Jar number.	Age of plants.	Nitrate applied.		Average height.	Average weight.	Hydrocyanic acid (HCN).	
			Per jar.	Per acre.			Per cent.	Per plant.
		Days.	*Gm.*	*Lb.*	*Cm.*	*Gm.*		*Mgm.*
J	35	69	None.	None.	36	1.0	0.0033	0.033
J	36	69	1.25	314	56	5.5	.0627	3.448
K	37	89	None.	None.	30	1.0	None.	None.
K	38	89	2.00	502	51	4.0	.1360	5.240

In the fourth column of Tables I, II, and III, is given the amount of nitrate added from the beginning of the experiment. As the nitrate was added in successive doses while the plants were growing, the amount present at any time was less than the total reported, since the plants had used some of it, and, further, there may have been some denitrification. The intention was to compare plants grown with scanty, moderate, and abundant supplies of nitrate, and the analyses reported indicate that the hydrocyanic acid increased with the amount of nitrate supplied, up to amounts beyond those usually considered advisable for field crops.

A third planting was made in April, using fresh Thurston loam subsoil in wooden boxes, 1 foot square, and 8 inches deep. Muriate of potash, at the rate of 300 pounds per acre, and treble superphosphate, 600 pounds per acre, were stirred into the surface of the soil, the seed planted on April 2, and the sodium nitrate given in a single application five days after the planting, at which time the plants were about 1 inch high. Each treatment was in duplicate and in some cases in quadruplicate, and the growth and appearance of all the plants with each treatment were very uniform. This series gave the most concordant results of any, probably because of the more favorable time of the year for growth, April 2 to May 20. Both the size of the plants and the percentage of hydrocyanic acid increased throughout the series, but the plants in the boxes that were given 760 pounds of nitrate per acre showed a slightly less thrifty appearance than those with only 570 pounds (Table IV).

TABLE IV.—*Sorghum plants grown on Thurston loam subsoil with various amounts of nitrate, given in a single application*

Lot.	Box number.	Age of plants.	Nitrate applied.		Average height.	Average weight.	Hydrocyanic acid (HCN).	
			Per box.	Per acre.			Per cent.	Per plant.
		Days.	*Gm.*	*Lb.*	*Cm.*	*Gm.*		*Mgm.*
L...	39	33	None.	None.	28	1.0	None.	None.
L...	40	33	0.50	47.5	30	1.2	None.	None.
L...	41	33	1.00	95.0	35	2.6	0.0160	0.42
L...	42	33	2.00	190.0	51	4.0	.0330	1.32
L...	43	33	4.00	380.0	48	4.0	.0810	3.24
L...	44	33	6.00	570.0	53	3.0	.0830	2.50
L...	45	33	8.00	760.0	46	4.0	.1200	4.80
M...	46	47	None.	None.	28	1.0	None.	None.
M...	47	47	.50	47.5	41	1.5	None.	None.
M...	48	47	1.00	95.0	41	3.0	.0025	.075
M...	49	47	2.00	190.0	48	4.0	.0037	.148
M...	50	47	4.00	380.0	58	6.0	.0140	.640
M...	51	47	6.00	570.0	66	8.5	.0290	2.460
M...	52	47	8.00	760.0	69	8.5	.0540	4.600

A qualitative test was made, using much smaller samples than those used in the preceding experiments, in order to determine whether a negative qualitative test might have resulted even from plants containing a considerable amount of hydrocyanic acid, because too small a sample had been employed. As 15 or 20 gm. is about the smallest sample that can be conveniently ground through the food chopper, these small samples of 2 or 3 gm. each were crushed in an agate mortar with a pestle. The plants tested were grown on Merrimac Loamy Sand, with nitrate added, and were dark green in color. They were from 20 to 28 cm. in height, and about 1 gm. in weight each. Two plants were used for each sample. In the analysis of all four samples, a satisfactory blue color resulted, about equal to that from 0.2 mgm. of potassium cyanid. It thus seems probable that working with very small plants, provided the sample is representative and thoroughly crushed or ground and adequately autolyzed, the failure of even a small sample (5 gm.) to give good qualitative test should be looked upon as indicating a probable lack of nitrate in the soil.

Although it has been generally recognized that the content of hydrocyanic acid is much greater in the leaves of sorghum than in the stems, it was not deemed safe to rely on analyses of the leaves only, but in the case of larger plants, the leaves and stems were analyzed separately and the hydrocyanic acid in the whole plant computed from these separate analyses. In the case of young small plants, it is difficult to separate leaf and stem; hence in those cases the whole plant was analyzed as one sample.

A few of these separate analyses of leaf and stem are reported in Table V to show that there is no characteristic difference in distribution of the hydrocyanic acid between fertilized and unfertilized plants, at least at the ages taken. The amount increases with the amount of nitrate supplied, and the percentage is usually several times as great in the leaves as in the stem.

If the plant is employed as an indicator, it may be found that analysis of the leaves only will give as correct an indication of the readily available nitrogen in the soil as analysis of the whole plant.

TABLE V.—*Distribution of hydrocyanic acid between leaf and stem of the same plant*

Sample number.	Age of plant.	Soil.	Nitrate added per jar.	Hydrocyanic acid content.		
				Leaves.	Stem.	Whole plant.
	Days.		*Gm.*	*Per cent.*	*Per cent.*	*Per cent.*
107	69	Coloma sand	0.75	0.1510	0.0221	0.0627
108	71	Merrimac loamy sand	None.	None.	None.	None.
109	71	do	0.75	.1111	.0062	.0369
110	92	do	None.	.0040	Trace.	.....
111	92	do	1.00	.1150	.0088	.0417
127	88	Thurston loam subsoil	1.50	.0610	.0100	.0340
129	88	do	1.25	.0180	Trace.	.0080
134	.....	Coloma sand	.....	.2697	.0519	.1360

SUMMARY

The hydrocyanic acid was determined in sorghum plants grown in the greenhouse, using three Minnesota soils low in nitrogen and adding sodium nitrate in different amounts. The size of the plants, their color and prussic acid content were all affected by the amount of nitrate applied.

In general, the percentage of hydrocyanic acid in the green plants was in proportion to the nitrate used. The effect on the prussic acid content continued even beyond the point where nitrate ceased to affect the color and size of the plants. In the light-colored sorghum plants, yellow to yellowish green, the percentage of prussic acid was very low, and in some cases none could be detected, while in all darker colored plants it was readily determined, even single plants weighing only 2 gm. giving distinct qualitative evidence. The leaves of the darker-colored plants contained several times as high a percentage of prussic acid as the stems, and the applications of nitrate showed no distinct effect upon its distribution between stem and leaf.

Sorghum gives promise of being useful as an indicator plant in studies of the supply of readily available nitrogen in soils. It responds readily not only by a more rapid growth and darker color but also by an increased hydrocyanic-acid content, which is highest in the young plants. As analysis requires only small samples; these may be had within a few weeks after the seed is planted.

THE COURSE OF ACIDITY CHANGES DURING THE GROWTH PERIOD OF WHEAT WITH SPECIAL REFERENCE TO STEM-RUST RESISTANCE[1]

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INTRODUCTION

Acidity measurements of juice expressed from wheat at frequent intervals from the early-seedling to the late-flowering stage have shown the occurrence of an interesting sequence of changes during the development of the plant. As the investigation was initiated for the study of the problem of disease resistance, the data were obtained on a number of varieties representing extremes of resistance and susceptibility to stem rust (*Puccinia graminis tritici* Erikss. and Henn.). The results therefore not only give evidence from a new standpoint of changing metabolic processes during the life of the wheat plant, but also permit conclusions on the question of a relation between acidity and resistance to stem rust.

PROCEDURE

The plants were grown in the greenhouse, although occasional checks were made with field-grown plants. The six varieties studied were sown, cut, and handled in pairs, each pair consisting of one relatively resistant and one relatively susceptible wheat. These pairs were the same in each of the three series, namely, Kota and Preston, Pentad (D–5) and Marquis, Khapli emmer and Little Club, respectively.

The same methods were followed for expressing and handling the juice and making the acidity determinations as were described in a preceding paper (*4*).[3] Both hydrogen-ion and titratable-acid concentrations were determined electrometrically. Immediately following the determination of the hydrogen-ion concentration of a sample, which in each case consisted of 10 cc. of undiluted juice, the electrometric titration was made on the same sample by adding N/20 sodium hydroxid solution, 1 cc. at a time, by means of a burette, the tip of which was inserted through the cork of the electrode vessel. The P_H values calculated from the successive potential differences so obtained were plotted against the volumes of sodium hydroxid required to produce them. From the resulting curves, the volume of alkali required to bring the reaction to P_H 8.3, the turning point of phenolphthalein, was determined and taken to represent the titratable acidity of the sample. Uniform procedure in the handling of each sample, together with the proper reversal of the sequence of determinations, reduced the chance of error to a minimum and insured the validity of comparisons.

[1] Received for publication Feb. 7, 1924.
[2] The writer is indebted to Dr. H. B. Humphrey, Dr. C. R. Ball, and Dr. H. Hasselbring for their helpful criticisms of the manuscript.
[3] Reference is made by number (italic) to "Literature cited," p. 735.

RESULTS

TITRATABLE-ACID CONCENTRATION IN RELATION TO STAGE OF DEVELOPMENT

As might be expected in view of the high buffer content of plant juices, more striking changes with the plant's development were found to occur in the concentration of titratable acid than in the concentration of free hydrogen ions in the juices from these plants. The titration results therefore will be presented first, though the hydrogen-ion determinations are not without interest and will be given in the section following.

Seed of the first series (A) of plants to be grown to maturity was sown on November 1, 1922,[4] in a greenhouse bench containing limed sandy-loam soil of slightly alkaline reaction (P_H 7.2). Acidity determinations were made on sap of each variety at frequent intervals from the time the plants were about 3 inches high until they were in the flowering stage at the age of approximately six months. After that time the measurements had to be discontinued because the plants became so dry that the requisite quantity of juice could not be expressed. The course of the changes in the titratable acidity of all six varieties during this period is shown in figure 1. The age of the plants was always reckoned from the date of sowing. Owing to the diurnal acidity changes reported to occur in many

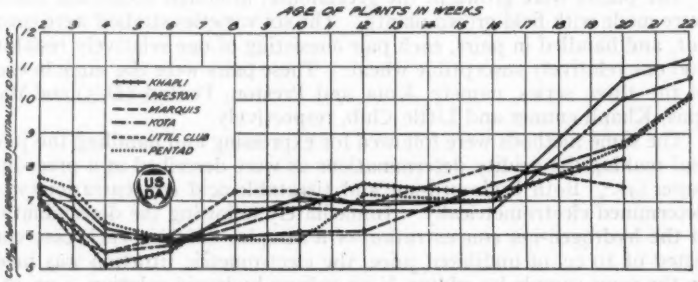

FIG. 1.—The titratable-acid concentration of wheat juice from plants of Series A, from the seedling to the late-flowering stage. (Greenhouse, Nov. 1, 1922, to Apr. 3, 1923.)

plants, only values obtained from plants cut about 1 o'clock in the afternoon were used in order to insure direct comparability of all points on the curves.

The first experiment was repeated with portions of the same seed lots sown three weeks later on another bench of the same greenhouse. The additional precaution was taken of making smaller plots and duplicating the sowing of each variety in order to lessen the chance of error from inequalities in the light, temperature, or water supply along the bench. The plants of this series (B) were somewhat more vigorous than those of Series A because of more favorable temperature and moisture conditions, but their rate of development was practically the same. To eliminate the effects of cutting at different times of the day, only values obtained for plants cut at 9 o'clock in the morning are plotted in figure 2.

[4] Seed for Series A and B was obtained by J. H. Martin from the Agricultural Experiment Stations at Dickinson, N. Dak. (Kota, C. I. 5878, Preston, C. I. 3081, Pentad, C. I. 3322, Marquis, C. I. 3641), Akron, Colo. (Khapli, C. I. 4013) and Moro, Oreg., (Little Club, C. I. 4066).

The plants of the third series (C) were grown to determine whether the correlation shown by the graphs in figures 1 and 2 to exist between acidity of the juice and stage of development would appear if the plants were in a different environment. Seed of the same varieties was obtained from a different source [5] and sown on February 8 in another

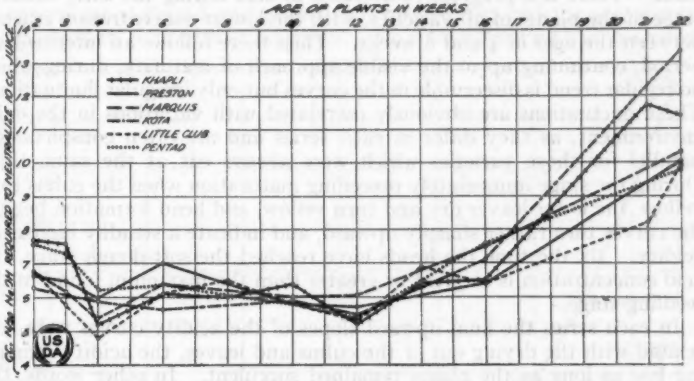

FIG. 2.—The titratable-acid concentration of wheat juice from plants of Series B, from the seedling to the late-flowering stage. (Greenhouse, Nov. 22, 1922, to Apr. 25, 1923.)

greenhouse having unlimed, sandy-loam soil with a reaction of P_H 6.8. The plants developed much more rapidly than those of the two preceding series. They reached the flowering stage in 14 to 16 weeks, whereas the plants of the same varieties in the fall-sown series had required 22 weeks to reach this stage. After 16 weeks, the plants no longer yielded the

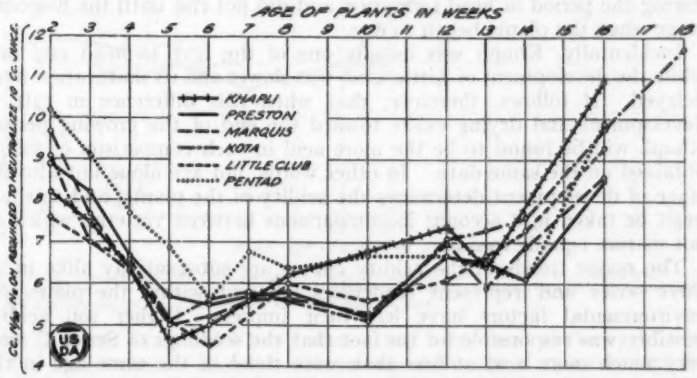

FIG. 3.—The titratable-acid concentration of wheat juice from plants of Series C, from the seedling to the late-flowering stage. (Greenhouse, Feb. 8 to June 14, 1923.)

requisite amount of juice for titration except in the case of Kota where one later measurement was made. Some of the acidity values plotted in figure 3 were obtained from plants cut in the morning and others from plants cut in the afternoon, as indicated in Table I.

[5] Seed of Kota, C. I. 5878, Preston 3081, Pentad C. I. 3322, and Marquis C. I. 3641, for Series C was sent by M. N. Levine from University Farm, St. Paul, Minn., and Khapli C. I. 4013 and Little Club C. I. 4066 by Dr. E. F Gaines, from Pullman, Wash.

The major trends in the curves of figure 3 are essentially the same as those of figures 1 and 2 and therefore establish the fact that the acidity of the wheat plant undergoes certain regular changes during development from the seedling stage to maturity and that these changes are not environmental effects but the results of inherent physiological characteristics. There is a progressive decrease in acid during the early-seedling stage of the plants of all varieties, with the lowest concentration reached between the ages of 4 and 6 weeks. Then there follows an intermediate period, continuing up to the visible approach of maturity, during which no regular trend is discernible in the curves but only irregular fluctuations. These fluctuations are obviously correlated with variations in the daily environment, as they differ in each series and are often conspicuously parallel for those varieties which were always cut at the same time. During the stage immediately preceding maturation when the culms turn yellow, the lower leaves dry and turn yellow, and head formation begins, the curves turn rather sharply upward, and indicate a steadily increasing acidity. By the time the heads have reached the soft-dough stage, the acid concentration is as great or greater than the maximum found in the seedling stage.

In each series the final upward slopes of the acidity curves were correlated with the drying out of the culms and leaves, the acidity remaining low as long as the plants remained succulent. In other words, the acid concentration during the period of head and flower formation is apparently determined by the loss of water from the tissues. For instance, in Series A and B the heads did not appear in the boot until the plants were partially dry, yet the acidity had begun to rise while the plants were still in the shooting stage. In Series C, on the other hand, in which development was more rapid and the head appeared in the boot while the plants were still vigorous and juicy, the acidity remained low during the period of head formation and did not rise until the flowering stage when the plants began to dry.

Incidentally, Khapli was usually one of the first to head and dry, while the development of Little Club was slower and its maturation more delayed. It follows, therefore, that when this difference in rate of development and drying exists toward the end of the growing period, Khapli will be found to be the more acid in each comparison of values obtained on the same date. In other words, not age alone but also the stage of development determines the acidity of the plants, and this fact must be taken into account in comparisons between varieties which do not mature equally rapidly.

The major trends in the acidity curves are substantially alike in all three series and represent tendencies inherent within the plant, yet environmental factors have left their impress. Higher soil acidity possibly was responsible for the fact that the seedlings of Series C were very much more acid at first than were those of the same age in the other two series. Also, environmental conditions, by determining the rate of development, determined both the duration of the interval elapsing before the beginning of the final ascending portion of each curve and the rate at which the acidity increased during the preripening period.

The foregoing data and discussion relate to healthy plants. When normal growth is prevented by an unfavorable environment, such as that which obtains for a winter wheat grown in the greenhouse during the warm spring months, the acidity curve differs from those given in

figures 1 to 3, inclusive. Figure 4 shows such a curve illustrating the acidity changes occurring in Kanred wheat sown on January 5, 1923, in a greenhouse which was too warm for winter wheat to grow normally. The plants were fairly vigorous in the early-seedling stage, but they were only 4 inches tall at the age of 10 weeks, 8 inches at the age of 13 weeks, and 9 inches at 15 weeks, at which time growth had almost ceased. The leaves remained turgid and green but very narrow, and the stems were very spindling, with almost no tillers. No sign of heading had appeared.

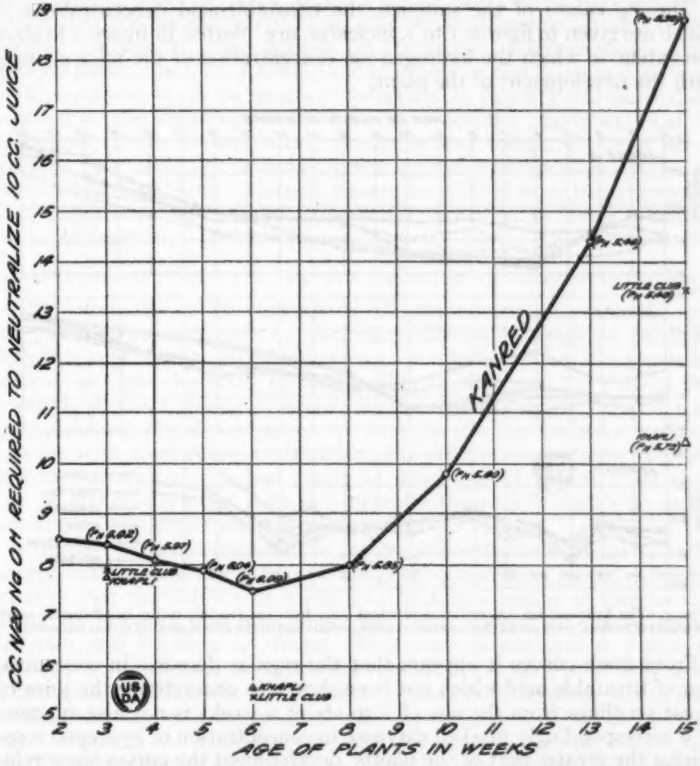

FIG. 4.—The titratable acidity (and P_H values in parentheses) of juice of unhealthy Kanred wheat from the seedling stage to cessation of growth, with some corresponding measurements on Khapli and Little Club growing alongside. (Greenhouse, Jan. 5 to Apr. 20, 1923.)

Seed of Little Club and Khapli emmer had been sown at the same time in the same bench. Khapli was flowering at the age of 15 weeks and the acidity measurements, made during the early-seedling, late-seedling, and flowering stages, respectively (fig. 4), were normal values for these stages of growth. Little Club, however, became badly infected with mildew, so that it never headed. By the age of 15 weeks its vigor was visibly affected by the disease, and its unhealthy condition was reflected in the abnormally high acid concentration for that stage of development.

The acidity of Kanred in this environment was very high compared to that of the spring varieties at the corresponding periods. The curve differs from those of the spring wheats in the very slight drop during the

seedling stage and in the steady extreme rise thereafter, coincident with the appearance of symptoms of stunted vegetative growth. The volume of N/20 NaOH required to neutralize a 10 cc. sample of juice increased from 8 to 18.8 cc. in seven weeks. Incidentally, the juice of these plants reached the hydrogen-ion concentration represented by P_H 5.39, which is extraordinarily high for wheat.

HYDROGEN-ION CONCENTRATION IN RELATION TO STAGE OF DEVELOPMENT

The P_H values of the samples, the titratable-acid concentrations of which are given in figures 1 to 3, inclusive, are plotted in figure 5 to show the extent to which the hydrogen-ion concentration of the juice changes with the development of the plant.

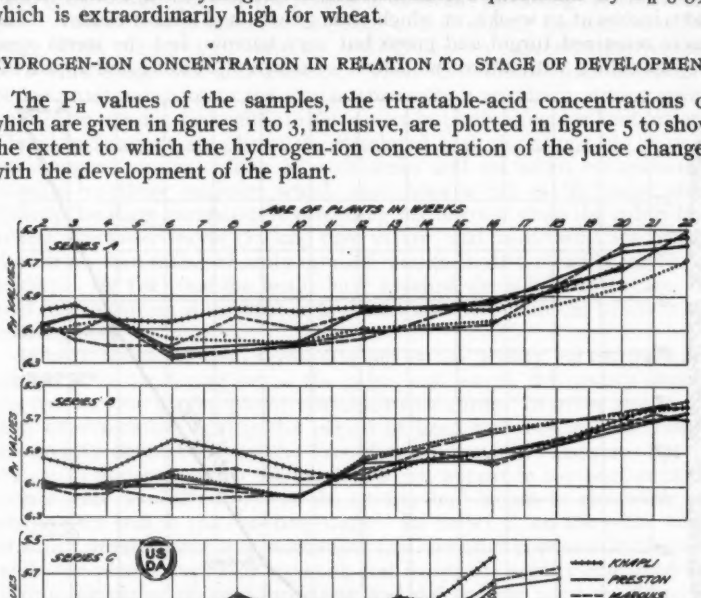

FIG. 5.—The hydrogen-ion concentration of wheat juice from the seedling to the late-flowering stage. The plants of Series C were cut in the forenoon at some periods and in the afternoon at others as indicated.

From these curves it appears that the regular decrease in concentration of titratable acid which has been shown to characterize the juice of wheat seedlings from the age of 2 to about 6 weeks is not accompanied by a correspondingly marked decrease in concentration of hydrogen ions. During the greater part of the plants' development the curves show relatively small and irregular variations which indicate the effects of daily fluctuations in the environment. During the preripening stage, characterized by an increasing titratable-acid concentration, there is a regular upward trend in each hydrogen-ion curve also, indicating that an increasing concentration of hydrogen ions parallels the increase in concentration of titratable acid.

The effects of minor daily fluctuations in environmental factors appear in many of the curves. The existence of these effects is made evident by the conspicuous parallelisms in the courses of certain curves, the corresponding points on which were obtained on the same days throughout. This does not mean that the varieties represented by such curves are more nearly like each other physiologically than like any others of the group, but simply that since the plants of each were always cut at the

same time and handled simultaneously, those external conditions affecting the acidity of one sample also affected that of the other in like degree. It is very interesting to find that the hydrogen-ion concentration of such morphologically unlike varieties as Khapli and Little Club and as Pentad and Marquis should fluctuate so similarly with small changes in environmental factors.

Evidence of the diurnal periodicity in the hydrogen-ion concentration of these plants appears in the curves of Series C in figure 5 and in the corresponding data in Table II from which the curves were plotted. The curves for all the varieties in this series fluctuate up and down together because at some periods all the acidity determinations were made on plants cut in the morning and at others on plants cut in the afternoon, as indicated on the graph. Reference to figure 3 will show that no effect of the difference in the hour at which the plants were cut is discernible in the corresponding titratable-acid values of these same samples. No such fluctuations appear in the hydrogen-ion curves of Series A and B of figure 5 because these were plotted from measurements all of which were obtained from plants cut at 1 p. m. in Series A and at 9 a. m. in Series B.

SIGNIFICANCE OF THE DATA FOR THE PROBLEM OF DISEASE RESISTANCE

The variability in the response of all wheat varieties except Khapli and Little Club to attack by the many different specialized races of stem rust now known (7) would seem to preclude *a priori* the responsibility of any one character, such as acidity. Moreover, the data of the preceding sections, giving evidence of extreme and regular variations in acidity during the development of each of the six varieties studied, together with what is known regarding the relative susceptibility of susceptible varieties at different stages of development, afford proof that high or low acid content does not influence the plant's ability to resist invasion by the stem-rust fungus.

A number of investigators have reported observations on the relative susceptibility of the wheat plant throughout its growing period. Farrer (1) says that the liability of wheat to rust attack begins as soon as the plant has flowered and ends when its foliage begins to change color. Freeman and Johnson (2) found wheat most susceptible to *Puccinia graminis* from the time the heads emerged from the boot until they were in full bloom. Melchers and Parker (5) used both heading plants and seedlings for the determination of varietal resistance of wheat to stem rust. Stakman and Piemeisel (8) state that cereals, including wheat, are usually susceptible at any age up to ripening time. Peltier (6) obtained infection of wheat by *P. graminis tritici* in the seedling, stooling, jointing, and heading stages, and his data indicate that the lowest degree of infection occurred in the seedling stage. So it seems obvious that the development of the fungus is not favored by the low acid concentration characterizing the post-seedling stage nor hindered by the relatively high concentration at flowering time.

Gassner (3) also, as the result of an extended experimental study of the relation between stage of development and degree of susceptibility to attack by *Puccinia graminis*, concludes that the susceptibility of wheat and other cereals increases with age, the most susceptible period occurring between the flowering stage and maturity. Equally interesting, in view of the fact that very young wheat seedlings are more acid than older plants, though usually not so acid as those which have passed the flower-

ing stage, is his observation that very young seedlings are sometimes more susceptible than older plants though not so readily infected as those which have passed the flowering stage. Thus it would appear that, if there be any correlation, the degree of infection of a susceptible variety varies directly rather than inversely with the acid concentration. However, these observations may be wholly unrelated and the apparent correlation accidental. At least it appears that the low acidity of the post-seedling period neither favors infection of susceptible varieties nor breaks down the resistance of resistant ones, nor does the high acidity of the older plants make susceptible varieties less liable to attack in the later stages of development.

The significance of the acidity data for the disease-resistance problem may be considered also from the standpoint of varietal comparisons at each stage of the plant's development. Pentad and Kota are resistant to specialized forms to which Preston and Marquis are susceptible. Khapli is resistant to all specialized forms known and Little Club is susceptible to them all (7). Yet at no period in the growth of the plants from the age of two weeks to maturity was either the titratable-acid or the hydrogen-ion concentration of the expressed juice related to the degree of resistance or susceptibility characterizing the variety. Comparisons are facilitated by the tabular arrangement in Tables I and II of the values from which the curves in figures 1, 2, 3, and 5 were plotted.

TABLE I.—*Titratable acidity of wheat at intervals from the seedling to the late-flowering stage, in cubic centimeters of N/20 sodium hydroxid required to neutralize 10 cc. of expressed juice*

SERIES A [a]

Age in weeks.	Kota.	Preston.	Pentad.	Marquis.	Khapli.	Little Club.
2	7.4	7.5	7.7	7.2	6.9	7.1
3	6.4	7.1	7.4	6.8	6.2	5.9
4	5.5	6.2	6.4	6.0	5.1	5.5
6	5.7	5.8	5.9	5.8	5.5	6.0
8	.....	.....	.....	.....	6.7	7.3
10	6.8	7.1	5.9	6.1	6.8	7.3
12	6.7	6.9	7.2	6.1	7.5	6.9
16	6.7	7.0	7.0	7.5	7.2	8.0
20	10.0	10.6	8.6	8.7	8.2	7.4
22	10.6	11.2	10.0	10.1	.....	.....
24	.....	.....	12.0	12.3	.....	.....
26	13.9	.....	.....	.....	.....	.....

SERIES B

2	6.3	6.7	7.6	6.8	7.7	6.8
3	.....	6.5	7.3	6.2	7.6	5.8
4	5.9	6.0	6.3	5.4	5.2	4.8
6	5.7	7.0	6.4	6.4	6.1	6.1
8	.....	.....	.....	.....	6.0	6.5
10	5.9	5.8	5.8	6.0	6.4	5.8
12	5.5	5.7	5.4	6.1	5.2	5.3
14	.....	.....	.....	.....	6.4	6.9
16	7.1	6.4	7.6	7.5	6.5	6.4
21	.....	.....	.....	.....	11.7	8.2
22	13.3	10.0	9.8	10.3	11.4	9.9
25	.....	.....	.....	11.6	.....	.....
26	.....	.....	.....	.....	.....	12.6

[a] Plants cut at 1 p. m., the others at 9 a. m.

TABLE I.—*Titratable acidity of wheat at intervals from the seedling to the late-flowering stage, in cubic centimeters of N/20 sodium hydroxid required to neutralize 10 cc. of expressed juice*—Continued

SERIES C

Age in weeks.	Kota.	Preston.	Pentad.	Marquis.	Khapli.	Little Club.
2	[a] 9. 1	[a] 8. 9	[a] 10. 2	[a] 9. 7	[a] 8. 2	[a] 7. 6
3	[a] 7. 0	[a] 8. 2	[a] 9. 1	[a] 7. 7	[a] 7. 5	[a] 7. 0
4	6. 3	6. 7	7. 7	6. 4	6. 4	6. 6
5	[a] 5. 1	[a] 5. 7	[a] 6. 9	[a] 5. 0	[a] 4. 6	[a] 5. 1
6	5. 6	5. 5	5. 6	5. 2	5. 0	4. 5
7	[a] 5. 8	[a] 5. 7	6. 8	5. 6	.....	.....
8	5. 6	5. 8	6. 4	5. 7	6. 2	6. 0
10	[a] 5. 1	[a] 5. 2	6. 7	5. 3	6. 8	5. 6
12	7. 1	6. 7	6. 7	7. 0	[a] 7. 4	[a] 6. 1
13	7. 3	6. 5	.....	5. 9	6. 4	6. 7
14	[a] 7. 6	[a] 6. 1	[a] 6. 1	[a] 6. 9	.....	[a] 7. 3
16	9. 8	8. 5	[a] 9. 9	[a] 8. 6	10. 9	9. 3
18	11. 7	.....	.....	.....	.....	.....

TABLE II.—*P_H values of the expressed juice of wheat at intervals from the seedling to the late-flowering stage*

SERIES A[a]

Age in weeks.	Kota.	Preston.	Pentad.	Marquis.	Khapli.	Little Club.
2	6. 10	6. 08	6. 12	6. 07	5. 98	6. 07
3	6. 12	6. 02	6. 07	6. 02	5. 95	6. 16
4	6. 03	6. 02	6. 04	6. 01	6. 04	6. 19
6	6. 27	6. 21	6. 15	6. 25	6. 05	6. 19
8	.....	.....	.....	.....	5. 97	6. 02
10	6. 18	6. 17	6. 17	6. 19	5. 99	6. 09
12	6. 11	5. 98	6. 09	6. 15	5. 96	5. 99
16	6. 06	5. 94	6. 05	5. 91	5. 98	5. 93
20	5. 59	5. 63	5. 84	5. 73	5. 72	5. 81
22	5. 54	5. 59	5. 68	5. 50	.....	.....
24	.....	.....	5. 57	5. 53	.....	.....
26	5. 58	.....	.....	.....	.....	.....

SERIES B

2	6. 11	6. 07	6. 11	6. 09	5. 94	6. 11
3	.....	6. 12	6. 09	6. 10	5. 99	6. 16
4	6. 12	6. 08	6. 11	6. 11	6. 01	6. 15
6	6. 19	6. 05	6. 03	6. 10	5. 83	6. 01
8	.....	.....	.....	.....	5. 90	6. 00
10	6. 16	6. 17	6. 17	6. 17	6. 01	6. 06
12	6. 00	5. 96	5. 93	5. 95	6. 06	6. 03
14	.....	.....	.....	.....	5. 94	5. 89
16	5. 90	5. 95	5. 76	5. 78	5. 90	5. 97
18	.....	.....	.....	.....	5. 83	.....
21	.....	.....	.....	5. 62	5. 62	5. 66
22	5. 66	5. 66	5. 62	5. 58	5. 62	5. 70
26	.....	.....	.....	.....	.....	5. 69

[a] Plants cut at 1 p. m., the others at 9 a. m.

TABLE II.—*P_H values of the expressed juice of wheat at intervals from the seedling to the late-flowering stage*—Continued

SERIES C

Age in weeks.	Kota.	Preston.	Pentad.	Marquis.	Khapli.	Little Club.
2	[a] 6.01	[a] 6.03	[a] 5.96	[a] 5.94	[a] 5.98	[a] 6.04
3	[a] 6.06	[a] 5.99	[a] 5.96	[a] 5.98	[a] 5.95	[a] 6.04
4	5.92	5.91	5.89	5.92	5.96	5.97
5	[a] 6.17	[a] 6.12	[a] 6.01	[a] 6.03	[a] 6.06	[a] 6.14
6	5.93	5.93	5.98	5.96	5.93	6.01
7	[a] 6.03	[a] 5.95	5.99	5.97	.....	.....
8	5.89	5.82	5.89	5.90	5.87	5.84
10	[a] 6.13	[a] 6.00	5.91	5.97	5.93	5.91
12	5.94	5.90	5.94	5.95	[a] 5.98	[a] 5.98
13	5.96	5.95	.....	6.02	5.84	5.92
14	[a] 6.02	[a] 6.10	[a] 6.05	[a] 6.08	[a] 5.88	[a] 5.97
16	5.74	5.78	[a] 5.85	[a] 5.82	5.60	5.77
18	5.67	5.73	.....	.....	.....	.....

[a] Plants cut at 1 p. m., the others at 9 a. m.

With the exception of the relatively high titratable-acid concentration of the young seedlings of Pentad in Series C, and the relatively high hydrogen-ion concentration of Khapli, up to the age of about 10 weeks, in Series A and B, the values in Tables I and II give no evidence of significant varietal differences, but rather of surprisingly close agreements for varieties so unlike, both as to morphological type and susceptibility to disease. Since these few varietal differences do not occur in all three series they probably represent differences in the reaction of the variety to the particular environment and are without significance for the disease-resistance problem. The data as a whole confirm the earlier conclusion (*4*) that the hydrogen-ion concentration of wheat juice bears no relation to varietal resistance to stem rust, and permit the same conclusion with respect to titratable-acid concentration.

CONCLUSIONS

The titratable acidity of the juice of the wheat plant undergoes a regular sequence of changes during development from the seedling stage to maturity. There is a progressive decrease, sometimes to half the initial concentration, between the ages of two and about six weeks. This period is followed by a period of relatively low acidity, with minor fluctuations, extending up to the visible approach of maturity, at which time the acid concentration rises as the plants ripen and dry. The final value may be twice the highest seedling concentration and almost three times that of the least acid stage.

The hydrogen-ion concentration of the juice of the wheat plant does not decrease appreciably between the ages of two and six weeks. It is greatly increased during the preripening period and reaches a relatively high value at the flowering stage and later.

This increasing acid concentration during the final stages of growth is correlated with the rate of drying rather than with head formation or kernel development.

Both the titratable-acid and hydrogen-ion concentrations are influenced by environmental conditions which determine the rate of growth and

which bring about daily fluctuations in acidity, but these variations are insignificant in comparison with the changes correlated with the changing stage of development of the plant, so that the major trends in the acidity curves persist clearly under all conditions.

Stunted, slow-growing plants, such as those of Kanred in a warm greenhouse, are characterized by an extremely high titratable-acid and hydrogen-ion concentration and may not have the intermediate period of low acidity. Also, infection by mildew, when severe enough to visibly affect the vigor of the plant, results in an abnormally high acidity.

The validity of varietal comparisons based on acidity measurements may be open to question unless the plants are at the same stage of development as well as of the same age, equally vigorous, and subjected throughout their development to the same environmental conditions.

Varietal resistance to stem rust is not related at any stage of development to titratable-acid or hydrogen-ion concentration.

High acidity of the juice does not hinder attacks of the stem-rust organism, for investigators have found that the wheat plant is as susceptible during the heading and flowering stages as it is during the earlier periods of low acidity. Conversely, low acidity does not predispose to the disease, since the plant is no more liable to infection during the period of lowest acidity than it is during the earlier and later stages. Moreover, resistant varieties pass through the period of low acid concentration at the same stage as do the susceptible ones and no breakdown in their resistance at this time has been reported.

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CYTOLOGICAL STUDIES OF DIPLOID AND POLYPLOID FORMS IN RASPBERRIES[1]

By ALBERT E. LONGLEY, *Cytologist, Office of Biophysical Investigations*, and GEORGE M. DARROW, *Pomologist, Office of Horticultural Investigations, Bureau of Plant Industry, United States Department of Agriculture*

This investigation of the raspberries of the subgenus Idaeobatus[2] is a continuation of cytological studies of the pollen-mother-cell development of Rubus begun by the senior author in 1921. In the first investigation, a report of which is now in press, the senior author found that diploid forms, that is, forms with a reduced number of seven bivalent chromosomes, were the exception in the subgenus Eubatus.

The present article presents the result of a careful study of the pollen-mother-cell development of species and varieties used in raspberry breeding by the Office of Horticultural Investigations of the United States Department of Agriculture, in which it is shown that most raspberries have seven bivalent chromosomes.

The study was undertaken to determine the chromosome number of the various species of raspberries as an aid to breeding and especially to find some explanation for the peculiar behavior of hybrids of La France and Ranere (St. Regis) varieties. From 100 or more seedlings of this particular cross only one weak plant gave well-developed fruit. A large percentage (32) bore sterile flowers and the remainder showed a range in fertility from those having an occasional flower in which a drupelet set to those with several drupelets to each flower. La France has been considered a variety of *Rubus idaeus*, the European raspberry, and Ranere, a variety of *R. strigosus*, the American red raspberry. These species are more closely related than many others which, when hybridized, give all, or nearly all, fertile progeny. For example, hybrids of Gregg (*R. occidentalis*) × Ranere (*R. strigosus*) in the same environment as La France × Ranere hybrids are entirely fertile. Certain other forms, when crossed, also gave an unexpectedly large percentage of infertile seedlings. Such results are difficult to interpret and it was hoped that knowledge of chromosome behavior might help to explain them.

The subgenus Eubatus (blackberries) in which the haploid number is seven has many chromosome groups, that is, tetraploid, pentaploid, hexaploid, and octaploid (*12*). The closely related genus Rosa also has the same basic chromosome number seven and has similar chromosome groups (*19*) (*3*) (*14*). Hybrids between polyploidal forms of Rosa are reported to behave in a manner quite different from the simple Mendelian expectation. It was considered possible, therefore, that there were similar polyploidal groups among the raspberries which would explain the results observed. To make the work as systematic, compre-

[1] Received for publication Feb. 9, 1924.
[2] The classification used in this paper is, for the most part, that of Focke given in "Species Ruborum" (*8*).
[3] Reference is made by number italic to "Literature cited," p. 747-748

hensive and helpful as possible, representatives of as many types of raspberries as could be procured were selected with special reference to the economic importance of the groups, their botanical relationship, and their possible value in breeding.

MATERIAL AND METHODS

The plants from which buds were taken were grown at the Bell Horticultural Field Station, Glendale, Md., and at Arlington Experiment Farm, Rosslyn, Va.

Material was collected during the last two weeks of May, in June, and again in September and October (1923). Buds were killed in weak chromo-acetic solution, and in Bouin's killing mixture, both of these being quite satisfactory.

All material was imbedded in nitrocellulose, this medium having been found very satisfactory for this type of investigation. The buds were sectioned about 15 micra thick so as to have uncut nuclei for study.

Heidenhain's haematoxylin was used for staining chromosomes. The mother cells were studied by the use of 2 mm. and 1.6 mm. Zeiss apochromatic lenses and No. 12 and 18 compensation oculars.

Pollen counts were made from anthers that had not dehisced. Plate 1, A, shows a bud in the stage of development usually chosen. The anthers were crushed in water on a glass slide and all plump grains counted as good. As a rule, the count was made from 100 grains only and the percentage of good pollen is, therefore, only approximate. Germination tests and special technique are necessary for a final test.

HISTORICAL AND CYTOLOGICAL DESCRIPTION

For convenience, the classification of Focke has been followed, for the most part, and the results have been presented in sections and series as given in "Species Ruborum" (*8*). The few polyploid forms, however, have been segregated and discussed after the review of the diploid forms.

Brief historical descriptions of many raspberry species and garden varieties are first given. The inconsistency existing in the nomenclature of horticultural raspberry and blackberry forms makes it necessary to include the available history of each species and variety used in this study, since in this way the identity of the material will be established more definitely. These descriptions, which we regret are incomplete, are followed by the results of morphological and cytological studies of pollen and pollen formation.

Table I presents a summary of the pollen counts and the chromosome number and behavior during the pollen tetrad formation of the species and varieties studied. Plates 2 and 3 show drawings of heterotypic prophases selected from type species, varieties, and hybrid forms.

TABLE I.—*Chromosome number and pollen condition of raspberries*

	Percentage of good pollen.	Number of haploid chromosomes.	Behavior of chromosomes at meiosis.	Plate No.
Subgenus Idaeobatus:				
Section Rosaefolii—				
1. *Rubus illicebrosus*	97	7	Regular	
2. *R. thunbergii*	86			
Section Pungentes—				
3. *R. lasiostylus*		7	do	2 H
4. *R. lasiostylus dizygos*×*R. idaeus* horticultural variety Superlative	40	7	do	2 K
Section Idaenthi—				
Series Nivei—				
5. *R. coreanus*		7	do	2 I
6. *R. coreanus*×*R. strigosus* horticultural variety Newman		7	do	2 L
7. *R. phoenicolasius*		7	do	2 O
Series Thrysidaei—				
8. *R. adenophorus*		7	do	2 P
9. *R. innominatus*		7	do	2 J
10. *R. innominatus*×*R. idaeus* horticultural variety Superlative		7	do	2 M
11. *R. innominatus*×(*R. idaeus*×*R. strigosus*) horticultural variety Cuthbert [a]	33	7	do	2 N
Series occidentalis—				
12. *R. occidentalis*—				
Horticultural variety Cumberland		7	do	2 C
Horticultural variety Farmer		7	do	
Horticultural variety Gregg	98	7	do	
13. *R. occidentalis* × *R. idaeus*—				
Horticultural variety Gregg × Wisbeck		7	do	2 E
Horticultural variety Royal		7	do	
14. (*R. occidentalis*×*R. idaeus*)×*R. strigosus*—				
Horticultural variety Cardinal	75	7	do	
Horticultural variety Royal×Newman		7	do	2 G
15. *R. occidentalis*×*R. strigosus*—				
Horticultural variety Gregg×King [b]	93	7	do	2 F
Series Eu-Idaea—				
16. *R. mesogaeus*	89	7	do	2 Q
17. *R. idaeus*—				
Horticultural variety Lloyd George	99	7	do	2 B
Horticultural variety Magnum Bonum				

[a] The classification of raspberry varieties used is that of Darrow; see literature cited (6).
[b] The pollen count was made from one of the most fertile forms of this cross.

TABLE I.—*Chromosome number and pollen condition of raspberries*—Continued

	Percentage of good pollen.	Number of haploid chromosomes.	Behavior of chromosomes at meiosis.	Plate No.
Subgenus Idaeobatus—Continued.				
Section Idaenthi—Continued.				
Series Eu-Idaea—Continued.				
18. *R. idaeus* (?)—				
Horticultural variety La France	56	14	Irregular	3 B
Horticultural variety Merveille Rouge	24	14	...do	3 E
Horticultural variety Merveille de Quatre Saisons Rouge	28	14	...do	3 F
Horticultural variety Surpasse Merveille a blanc	10	14	...do	3 G–I
Horticultural variety Souvenir de Desire Bruneau	36	.....	.....	.....
Horticultural variety All Summer	97	21/2	...do	.....
Horticultural variety White Queen	96	21/2	...do	.....
19. *R. idaeus*×*R. strigosus*—				
Horticultural variety Cuthbert	70	7	Regular	2 D
Horticultural variety Marlboro	50	.....	.....	.....
20. *R. Idaeus*×*R. strigosus* (?)—				
Horticultural variety La France×Ranere	Variable.	21/2	Irregular	3 C
Horticultural variety Erskine	50	21/2	...do	3 D
21. *R. strigosus*—				
Wild Plant	100	7	Regular	2 A
Horticultural variety Eaton	.....	7	...do	.....
Horticultural variety King	.....	7	...do	.....
Horticultural variety Newman	.....	7	...do	.....
Horticultural variety Ranere	100	7	...do	3 A

DIPLOID RASPBERRIES

SECTION PUNGENTES × IDAENTHI

R. lasiostylus dizygos×*R. idaeus* horticultural variety Superlative. This cross was made by the junior author at the Bell Horticultural Field Station and buds were taken from a selected individual.

SECTION IDAENTHI

R. coreanus×*R. strigosus* horticultural variety Newman. This cross was made by the junior author at the Bell Horticultural Field Station and the buds taken from a selected bush. The *R. coreanus* parent of this hybrid was grown from seed secured from Kew, England, but itself seems to be a hybrid, *R. coreanus*×*R. biflorus*.

R. innominatus S. Moore. The plants of this species were propagated from plants grown by the late Dr. Walter Van Fleet, presumably from seed sent by E. H. Wilson from China.

R. adenophorus Rolfe. The plants of this species were obtained from the Office of Foreign Seed and Plant Introduction, United States Department of Agriculture, under No. 52939.

R. innominatus×*R. idaeus* horticultural variety Superlative. The plant from which buds were taken was one of several of this cross made by the junior author at the Bell Horticultural Field Station.

R. innominatus×(*R. idaeus*×*R. strigosus*) horticultural variety Cuthbert. Horticultural variety Van Fleet. This hybrid was made by the late Doctor Van Fleet at Chico, Calif., and the plants grown from seed at the Bell station.

R. occidentalis. Horticultural variety Cumberland. This was originated by David Miller of Camp Hill, Pa., and introduced in 1898.

Horticultural variety Farmer (Plum Farmer). This was found in a shipment of another variety received by L. J. Farmer, Pulaski, N. Y., and introduced by him in 1895. Farmer, as an early sort, and Cumberland as a late variety are the leading black raspberries grown in the United States.

R. occidentalis×*R. idaeus*. Horticultural variety Gregg×Wisbeck. The buds were collected from one of several seedlings of this cross made by the junior author at the Bell station.

Horticultural variety Royal. This variety was originated in Indiana by L. H. Gerton, and was introduced by L. J. Farmer, Pulaski, N. Y., in 1909.

(*R. occidentalis*×*R. idaeus*)×*strigosus*. Horticultural variety Cardinal. This variety originated on the place of A. H. Griesa, Lawrence, Kans., in 1888, apparently from seed of the Shaffer. Because of its glandular hairy inflorescence it is considered a cross of the Shaffer with a variety of *R. strigosus* (*4*).

Horticultural variety Royal×Newman. The material for study was taken from a plant growing at the Bell station which was one among the many crosses of this parentage made by the junior author.

R. occidentalis×*R. strigosus*. Horticultural variety Gregg×King. This material for study was taken from one plant, which was selected for its good fruit, from a large number of plants of this cross made by the junior author, at the Bell station. Most seedlings of this cross were partly sterile, setting but few drupelets to each flower. This seedling was selected as the best and its pollen count showed a notably high percentage of good pollen grains. The seed parent was a variety of black raspberry resembling Gregg but concerning the identity of which there was some question. The abundant glandular inflorescence clearly distinguishes these crosses from the Royal and the Gregg×Wisbeck.

R. idaeus. Horticultural variety Lloyd George. This variety was found wild and introduced by J. H. Kettle, Corfe Mullen, Winborne, Dorsett, England, in 1920.

Horticultural variety Magnum Bonum. This old variety of Europe was introduced into America about 1840. It differs markedly from the other European raspberries which we have studied. The turions are densely covered with glandular bristles and it resembles mountain and maritime forms.

R. idaeus×*R. strigosus*. Horticultural variety Cuthbert. This variety originated as a chance seedling in the garden of Thomas Cuthbert at Riverdale, N. Y., about 1865 growing near plants of the Hudson River Antwerp, a variety of *R. idaeus*. It is, therefore, considered a hybrid of *R. idaeus*×*R. strigosus*.

Horticultural variety Marlboro (*Abundance, Laxton's Abundance, Perfection of England*). The material used was gathered from plants received as Laxton's Abundance from Laxton Bros., England, a variety which was determined to be identical with Marlboro by Grubb (*9*). This is confirmed by our examination. Marlboro was originated by A. J. Caywood, Marlboro, N. Y., as a cross of Highland Hardy, and a seedling from English Globe and the Hudson River Antwerp (*5*), and introduced in 1884. Highland Hardy is supposed to be a variety of *R. strigosus*. November Abundance is probably a distinct sort, having originated in England.

R. strigosus Mchx. Wild plants from Hebron, N. Y. In September, 1920, selections were made of bushes fruiting freely in the fall on turion tips. These were transplanted to the collection of the Bell Horticultural Field Station.

Horticultural variety Eaton. Found as a chance seedling at Cambridge City, Ind., in 1885. Its glandular inflorescence refers it to *R. strigosus*.

Horticultural variety King. The history of this variety is not entirely clear. It is widely grown in the upper Mississippi Valley States. The buds used were from plants of this variety secured from Michigan. It is supposed to be the Thompson's King sent out by the Cleveland Nursery Co. of Rio Vista, Va., in 1892, which variety was grown from seed of Thompson by T. Thompson of Richmond. Beach, in 1895, however, states of this variety, "Canes vigorous, show evidence of *Idaeus* parentage" (*2, p. 204*). King, as grown to-day, does not show evidence of *R. idaeus* parentage, except perhaps in that a large percentage of its progeny are more or less infertile, but it has the glandular inflorescence, the thin leaves, and light-red fruit of *R. strigosus* and is very hardy. Over 100 hybrids with a black raspberry and many hundred other hybrids and crosses with it made by the junior author fail to show characteristics of *R. idaeus* but do show characteristics of *R. strigosus* and it is classified accordingly.

Horticultural variety Newman. This variety was grown from seed of Eaton, by C. P. Newman, La Salle, Quebec, and was introduced by the Provincial Government of Quebec, Canada, in 1921. Its hardiness and light red fruit refer it to this species. Newman states that the pollen parent may have been King.

Horticultural variety Ranere (*St. Regis*). The Ranere was found wild near Hammonton, N. J., by A. Ranere and was extensively grown by him and others several years before 1910 when it was introduced under the name "St. Regis." It is densely glandular, hairy, very hardy, has thin leaves and light red fruit, and is referred to *R. strigosus* (*6*).

Table I shows that all raspberries examined cytologically can be separated into two classes, those with seven haploid chromosomes, and those with more than seven.

Such a division also separates all species into two groups based on the distribution of the chromosomes during the reduction divisions of the pollen mother cell. The diploid species, that is, those showing seven bivalent or haploid chromosomes just previous to the first reduction division, have the somatic or univalent chromosomes pairing very promptly in the prophase of the first reduction division, and these bivalent chromosomes divide regularly and promptly in both the first and second divisions. Such a division gives a quantitatively equal distribution of all chromatin material to the four daughter nuclei, and consequently this type of division has been termed regular. However, a study of the reduction phases showed, in rare cases, a little tardiness in pairing of univalent chromosomes, or occasionally a single chromosome lagging behind its associates in reaching the pole after either the first or the second reduction division but these irregularities were so rare that they may be disregarded.

POLYPLOID RASPBERRIES

The following section is a discussion of a smaller group of horticultural varieties of raspberries in which the chromosome number is larger than is the rule in diploid forms and in which chromosomes show characteristic irregularities in their distribution during meiosis.

R. idaeus (?). Horticultural variety La France. This variety was secured from the introducer, John Scheepers Co., New York City. The original stock was introduced from France probably between 1890 and 1900 and grown at Stamford, Conn., from which place it was distributed. It resembles Merveille de Quatre Saisons Rouge very closely and may be identical with it.

Horticultural variety Merveille Rouge. This old French variety was raised and introduced by Simon Louis Frères of Metz, France. Our stock which was secured from Laxton Bros., Bedford, England, is very similar to Merveille de Quatre Saisons Rouge.

Horticultural variety Merveille de Quatre Saisons Rouge. This is probably the same as Perpetual de Billiard. It was raised and introduced in 1849 by M. Billiard, nurseryman of Fontenay, near Paris. Stock of this was obtained from Orleans, France. It is one of the old varieties and is reported as identical with October Red (*20*) and the Old Double-Bearing (*18*). It is also reported as a supposed seedling of Fastolff (*16*).

Horticultural variety Surpasse Merveille a Blanc. Stock under this name was secured from Orleans, France, but it bears red fruit and resembles Merveille de Quatre Saisons Rouge very closely and its probable identity with this variety is indicated further by this cytological study.

Horticultural variety All Summer. Plants of this variety were obtained from Orleans, France.

Horticultural variety White Queen. White Queen was introduced in 1920 by Wm. M. Hunt & Co., of New York City, who state that it may be of French parentage. It was found on the place of Jonathan Thorne at Black Rock, Conn., and is supposed to be a chance seedling. It fruits freely in the autumn on young canes.

La France was the first raspberry studied in which more than the 7 haploid chromosomes were found and, because of this, all phases of meiosis have been studied critically. Plate 3, B, pictures the 14 bivalent chromosomes. They appear crowded in the drawing, but are on the periphery of the nucleus and quite separate in the actual observations. Not only is there an increase in chromosome number above that of diploid forms, but there is also a change in the general appearance of the anthers. Clear figures, plump mother cells and prompt pairing of the chromosomes at diakinesis are absent and one has to study much material and stain carefully in order to overcome the unfavorable conditions found in this polyploid form during meiosis.

It seems unnecessary to discuss in detail the reduction divisions of this variety but one characteristic should be mentioned, that is, the irregular distribution of chromosomes, some of which lag on the spindle and are extruded from the daughter nuclei. Such behavior is believed to indicate hybrid origin, and results in the formation of pollen grains with varying chromosome numbers and varying in viability.

La France, though not hardy, endures the winters better than many European raspberry varieties. It has dark green foliage which is more resistant to leaf spot than spring fruiting European sorts. It also bears fruit quite freely on the turion tips in the autumn. Five varieties with characteristics similar to La France were selected and five additional polyploidous forms were found. The cytological study of these forms had associated with it the difficulties referred to in the foregoing variety. The mother cells frequently were thin and vacuolated and collapsed before they reached the tetrad stage. After much search favorable figures of many stages were found.

The 14 haploid chromosomes of Merveille Rouge and Merveille de Quatre Saisons Rouge are represented in Plate 3, E and F. These typical prophases resemble that pictured for La France and the later phases have associated with them the same interesting irregularities. Consequently, one would expect to find a large amount of pollen sterility, an expectation borne out by the actual observations, both varieties having only 25 per cent viable pollen.

Surpasse Merveille a Blanc has 14 bivalent chromosomes. The early prophases which showed the univalents grouped in pairs were very favorable for study. Plate 3, G was drawn from a figure that had only three bivalent chromosomes, the remaining 22 univalents are seen grouped in pairs. A later prophase showing only bivalent chromosomes is represented in Plate 3, H. Plate 3, I, is a drawing of a very late heterotypic

prophase or early metaphase. Seven chromosomes are at the equatorial plate, some even having divided, while the remaining 7 are lagging in their approach to the plate. It is these laggards that cause observed irregularities in this and the homotypic division. They are again tardy in their movement from the nuclear plate and may not be included in the daughter nuclei and so the resulting pollen grains do not receive either a qualitatively or quantitatively equal amount of chromatin material. This type of reduction is styled as irregular.

The chromosome number of varieties White Queen and All Summer seems to be $\frac{21}{2}$. During meiosis they show many of the irregularities peculiar to triploid hybrids, and, consequently, they are grouped with the foregoing polyploidous raspberries. The pollen of these two sorts, however, seems remarkably good when their chromosome condition is considered.

R. strigosus × *R. idaeus*, (?). Horticultural variety Erskine. This originated at Lee, Mass., with E. J. Norman, being found in 1895 among Marlboro plants set in 1890. Two other varieties, the Cuthbert and Golden Queen, were being grown by Norman at that time. As the plant was small, he supposed it to be a seedling of the Marlboro. The Marlboro plants were obtained from Ellwanger & Barry of Rochester, N. Y., who also were growing Fontenay and other European raspberries. Fontenay is an autumn-fruiting sort and probably belongs to this polyploid group. There is a bare possibility that Erskine may have come from that source. If the assumption that Erskine originated from the Marlboro is correct then it is the only polyploid variety of American origin derived from American varieties yet found.

Horticultural variety La France × Ranere. These hybrids were made by the junior author at the Bell Horticultural Field Station and two of the seedlings, one entirely sterile, the other nearly so, were selected for this study.

Erskine was the second polyploid raspberry discovered and difficulty was experienced in finding good mitotic figures to study the various phases of this form. This supposed hybrid is triploid, and the reduced chromosome number is represented in Plate 3, D. There are generally 10 chromosomes at diakinesis. In the earlier study of the Eubatus subgenus by the senior author, triploid forms were found to be very abundant, and this hybrid behaves in a manner very similar to that described for triploid blackberries. Seven of the bivalent chromosomes behave in a regular manner during the reduction phases, but the remaining chromosomes are slow about fusing in the heterotypic prophase. These laggards are distributed, during meiosis, in an irregular manner to the four daughter nuclei or are frequently extruded into the cytoplasm where they degenerate or become the nuclei for dwarf pollen grains.

The hybrid, La France × Ranere, is triploid, which would be the natural result if the parents contributed 14 and 7 chromosomes, respectively. Plate 3, A, B, and C, represents prophases of the parents and the hybrid. This opportunity to study an F_1 raspberry hybrid has shown that it behaves in a manner very similar to that described by Rosenberg (*15*) for the well-known Drosera hybrid. This artificially produced raspberry hybrid has all the irregularities of chromosome distribution noticed in triploid blackberries, and furnishes additional evidence that many of our blackberries are very recent hybrids.

In collecting material of this La France × Ranere cross, buds were gathered from two seedlings, No. 1 and No. 2. All anthers of No. 1, as pictured in Plate 1,C, were so sterile that it was impossible to find normal reduction phases. Such extreme sterility recalls the sterility of *Prunus cerasus* × *P. avium* as reported by Dutrochet (7) who writes, "The 'stamina'

formed a compact mass in which no pollen was formed." Form No. 2 was more fertile, as the anthers pictured in Plate 1, B, show. From this plant there was procured sufficient material for a study of the reduction division. Since this hybrid is triploid, it corresponds to the intergroup crosses of wheat (Triticum) in which Sax (*17*) found the greatest sterility. It indicates that the sex cell on La France side had 14 chromosomes. The behavior in egg formation, therefore, differs from that studied by Täckholm for the Canina group of roses. He found that the viable egg of a tetraploid species had 21 chromosomes and the viable pollen usually had 7 chromosomes.

It seems certain that this list of polyploid raspberries is not complete. The varieties Buckeye, Hailsham, and Souvenir de Desire Bruneau show external characteristics which place them in this group, that is, as grown in the United States, they are more hardy than the diploid varieties of *R. idaeus*, have heavy dark green foliage, resist leaf spot, and bear fruit on young canes in autumn.

DISCUSSION

POLLEN STERILITY IN RASPBERRIES

Table I shows a dozen diploid forms in which the per cent of good pollen has been determined. Five of these diploid varieties are hybrids, and the large amount of sterile pollen existing in them, in contrast to the small amount of sterile pollen that generally exists in stable species, supports the view that hybridizing may cause noticeable sterility in the offspring (*10*).

This table also shows a second group of sterile raspberries, including all but two of the polyploidous forms. The high percentage of good pollen found in the two exceptions was unexpected, and since a study of these varieties is not complete, no explanation will be attempted. The remaining six forms are very sterile and characterized by an unequal distribution of the chromosomes during the pollen tetrad formation. This extreme sterility may be attributed to unbalanced chromosome conditions existing in the nucleus of the pollen grain, which in turn is recognized as a character of hybrids between incompatible species. The inference, therefore, seems to be that raspberries showing pollen sterility are of hybrid origin and that polyploidous raspberries are the result of crosses between plants belonging to different chromosome groups.

CHROMOSOME MULTIPLICATION DUE TO HYBRIDIZATION

Polyploidy in raspberries seems to have originated in both Europe and America. Our knowledge is too limited to allow the acceptance of one explanation to the exclusion of all others as to how it originated, but the present data support the theory that polyploidism in raspberries has originated through hybridization.

In England *Rubus caesius*, a tetraploid trailing blackberry, blossoms at approximately the same season as the raspberries, while the common blackberries blossom several weeks later than the usual raspberry flowering season. A species with dark thick leaves, similar to *R. caesius*, seems essential to explain the characters present in polyploid raspberries. One English autumn-bearing raspberry was reported as a hybrid, *R. caesius* × *R. idaeus* (*11*). The junior author has raised many *R. idaeus* ×

R. caesius crosses from seed and obtained a great variety of forms, but unfortunately these F_1 plants had been destroyed previous to this study and no cytological material is available.

An F_3 hybrid, originated by Professor Ness (*13*) of the Texas Experiment Station, is a cross between the Cardinal raspberry, a diploid form, and *R. rubrisetus*, a southern blackberry. A cytological study of this hybrid seems to provide evidence to support the theory of the origin of polyploidism in raspberries through hybridization and selection.

Three figures drawn from early reduction phases of this F_3 hybrid show the cytological conditions in this hybrid. Plate 3, J and K, shows the 14 chromosomes of this tetraploid form, while Plate 3, L, pictures a slight irregularity in chromosome distribution. It was exceptional to find irregularities during meiosis, but the history of this hybrid shows that it has been selected through several generations and now behaves like a stable species.

This F_3 hybrid demonstrates that fertile raspberry-blackberry hybrids are possible and it also shows that a tetraploid Rubus has been experimentally produced with the characteristics of a stable species.

After studying many known raspberry × raspberry hybrids without finding a single case of chromosome multiplication it seems worth while to consider the possibility that all the polyploidous forms described in this article are the result of raspberry × blackberry hybrids that have been selected artificially or naturally and now have found a place in our commercial varieties of raspberries.

SIGNIFICANCE OF DIPLOID, TRIPLOID, AND TETRAPLOID RASPBERRIES

We have shown that all the species or varieties of raspberries examined have the basic chromosome number seven. Furthermore, hybrids even between species which are quite different in their external characters also have the same basic number seven. A few varieties referred to *R. idaeus*, however, are triploid or tetraploid and the presence of these few variant forms, in a group otherwise uniform in this respect, is important.

There are genera in which chromosome multiplication has been reported, and in which new hybrid species and varieties are appearing continually. The blackberries, roses, and hawthorns are well known examples where multiplication of species and varieties is associated with chromosome multiplication. In Europe several thousand Eubatus Rubi have been collected and described as species while the recent American publication on "Standardized Plant Names" (*1*), devotes 50 pages to listing varieties of roses alone. The presence of a few polyploid raspberries, of unquestionably recent origin, may be the beginning of a similar multiplication in raspberry forms. All polyploidous raspberries belong to the best autumn-fruiting European group, and, therefore, an effort should be made to combine by hybridization their good qualities with those of our hardy American varieties.

La France × Ranere hybrids, obtained at the Bell station by the junior author demonstrate the variable results that may be expected from intergroup hybrids. The raspberry breeder who attempts to introduce the characters present in polyploid raspberries by hybridization will find his efforts seriously limited by the incompatibility frequently existing between hybrids involving different chromosome groups.

The recent study of tetraploid Datura hybrids (*4*) is a carefully planned scientific research into the genetic behavior of a recently discovered

group of polyploid forms. This pioneer investigation shows that the chances of obtaining new combinations have increased with an increase in chromosome number and it has been found by studying large populations from such hybrids that the offspring follow certain genetic laws of segregation. It is only by means of such experimental hybridization that the plant breeder, who is working with a genus such as Rubus, may help to deduce laws of inheritance that will aid him to combine desirable qualities from the varied material at hand and segregate them in the later generations.

SUMMARY

Diploid species and hybrids are the rule in the Idaeobatis subgenus of Rubus.

Triploid and tetraploid forms are few in number but are a significant and characteristic group of raspberries.

This study suggests that polyploid raspberries are *Idaeobatis* × *Eubatus* hybrids.

Polyploidism, if once established in a group, is likely to increase; consequently the breeder attempting interchromosome group crossing will find his difficulties increased, the interpretation of results more difficult, but the chance of obtaining new combinations of characters multiplied.

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PLATE 1[a]

A.—Red raspberry buds and flowers. Pollen counts were made, for the most part, from anthers taken from buds at approximately the stage of development shown in the youngest bud.

B.—Photomicrograph of an anther from a La France×Ranere hybrid that was used for the study of the chromosome behavior in this triploid individual.

C.—Photomicrograph of anther from a sterile La Franc×Ranere hybrid.

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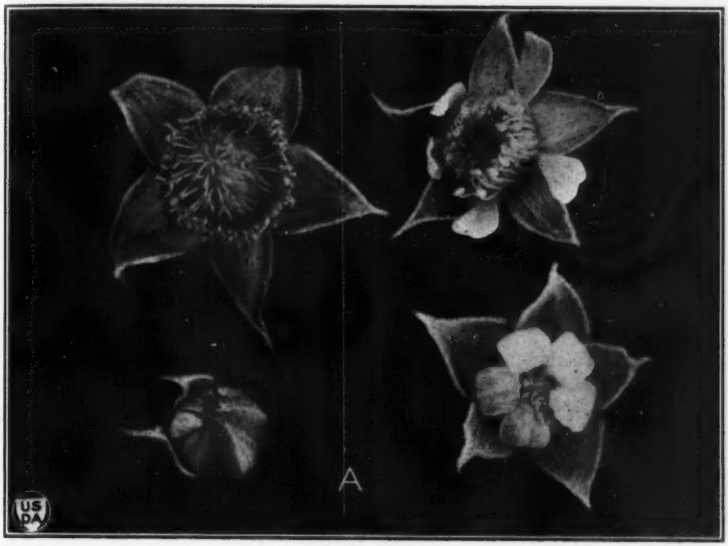
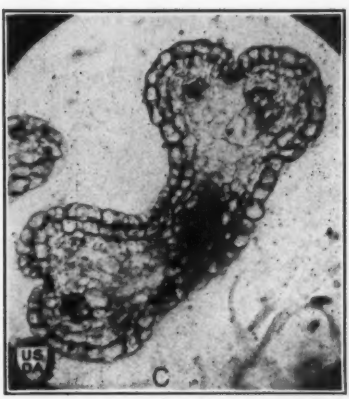
[a] The drawings of Plates 2 and 3 were made with a 2mm. Zeiss apochromatic lens, a No. 18 compensation ocular and a camera lucida. One exception, Plate 3, G, was made with a 1.7 lens and a No. 12 ocular. All drawings were made with the paper on the table level with the base of the microscope.


A

B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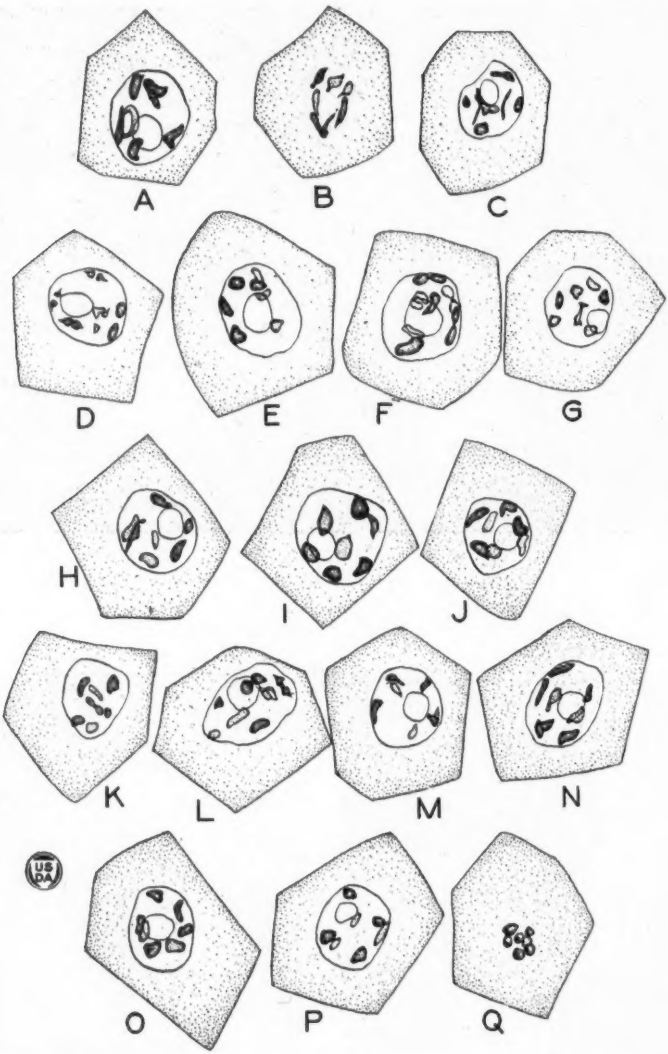
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PLATE 2

Pollen-mother-cells of rubi at diakenisis:[a]

A.—*Rubus strigosus*.
B.—*Rubus idaeus*. Horticultural variety Lloyd George (early heterotypic metaphase).
C.—*Rubus occidentalis*. Horticultural variety Cumberland, showing the two, long, slender chromosomes.
D.—*Rubus idaeus*×*strigosus*. Horticultural variety Cuthbert (early prophase).
E.—*Rubus occidentalis*×*idaeus*. Horticultural variety Gregg×Wisbeck.
F.—*Rubus occidentalis*×*strigosus*. Horticultural variety Gregg×King.
G.—*Rubus* (*occidentalis*×*idaeus*)×*strigosus*. Horticultural variety Royal×Newman No. 23.
H.—*Rubus lasiostylus*.
I.—*Rubus coreanus*.
J.—*Rubus innominatus*.
K.—*Rubus lasiostylus* variety *dizygos*×*idaeus* horticultural variety Superlative.
L.—*Rubus coreanus*×*strigosus* horticultural variety Newman.
M.—*Rubus innominatus*×*idaeus* horticultural variety Superlative.
N.—*Rubus innominatus*×(*idaeus*×*strigosus*) horticultural variety Cuthbert.
O.—*Rubus phoenicolasius*.
P.—*Rubus adenophorus*.
Q.—*Rubus mesogaeus*.

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[a] Drawings reduced one-half.

PLATE 3

Pollen-mother-cells of some polyploidous rubi:[a]

A.—*Rubus strigosus*. Horticultural variety Ranere, diploid.
B.—*Rubus idaeus* (?). Horticultural variety La France, tetraploid.
C.—*Rubus idaeus* (?)×*strigosus*. Horticultural variety La France×Ranere, triploid.
D.—*Rubus strigosus*×*idaeus*, (?). Horticultural variety Erskine, triploid.
E.—A tetraploid Rubus, Merveille de Rouge.
F.—A tetraploid Rubus, Merveille de Quatre Saisons Rouge.
G, H and I.—Three stages in the heterotypic division of Surpasse Merveille a blanc.
J, K and L.—Three stages in the heterotypic division of the Ness, a tetraploid raspberry-blackberry hybrid.

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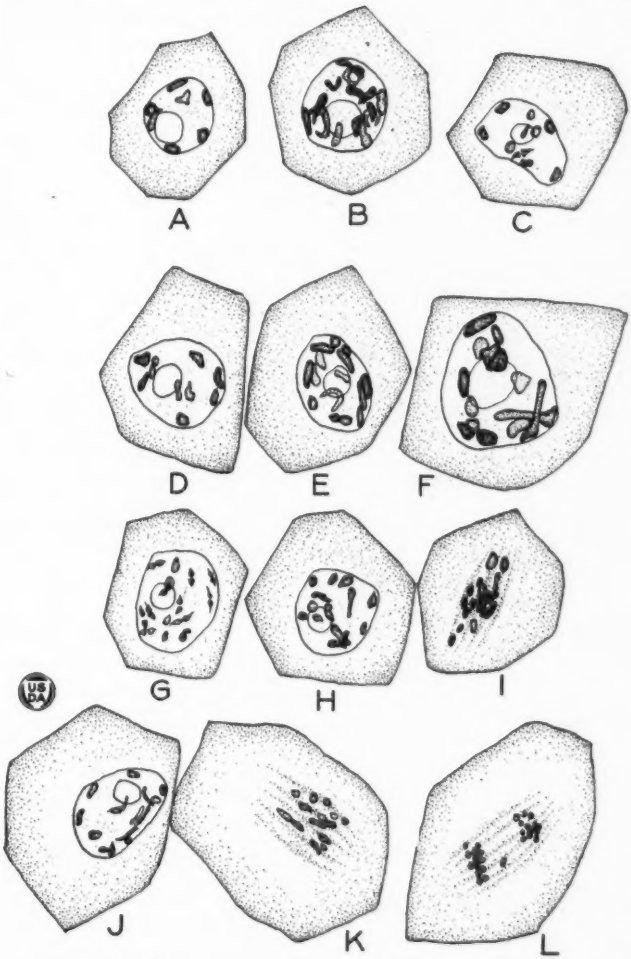
[a] Drawings reduced one-half.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AECIDIOSPORE DISCHARGE AS RELATED TO THE CHARACTER OF THE SPORE WALL[1]

By B. O. DODGE

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During the prosecution of cytological studies on species of rust, the writer has made some observations on the development of their aecidia and on the origin and discharge of aecidiospores. The formation of germ pores and the concomitant development of structures which play an important rôle in spore discharge from the aecidium of *Gymnosporangium myricatum* (Schw.) Fromme are briefly described in this paper. Attention is also directed to important differences obtaining with respect to these features in the orange-rusts of Rubus.

The mechanics involved in the discharge of spores by such fungi as Pilobolus have always been a fascinating subject for investigation. More recently Buller (*1*)[2] has added greatly to our knowledge of spore discharge by the Hymenomycetes, and has pointed out that in the common mushroom there is a very delicate adjustment of organs to insure the unobstructed fall of the spores between the gills as they come to maturity. The basidiospore is started off along its sporabolic trajectory with an initial horizontal velocity imparted it by the force of some miniature explosion, as it were. The actual mechanics of the process still remain a mystery. It is a well-known fact that the sporidia of Gymnosporangium are frequently set free from the promycelium with enough force to carry them well beyond the other promycelia on the telial horns. Dietel (*3*) has shown by measurements that sporidia of certain rusts are discharged from sterigmata with sufficient violence to carry them out horizontally nearly a millimeter.

Observations that have been made on the part played by the peristomal teeth of moss capsules or by the segments of the "peridium" of the Geasters in spore discharge have probably led us to assume that the hygroscopic action of peridial cells of the roestelia type serves as much to check the scattering of spores during unfavorable weather as it does to dislodge them.

In attempting to make stereoscopic photographs of the beautiful aecidia of *G. myricatum* with a binocular outfit, the writer (*5*) was not very successful in obtaining clear pictures of the spores in the cups, because the group of spores brought into focus generally disappeared before the completion of the exposure. On examining aecidia on freshly gathered leaves with a microscope of fairly low power, it was noticed that the spores were being shot out of the cups one by one with considerable force like popped corn jumping out of the popper. When recently matured aecidia were dissected out of the leaf and placed on slides in damp chambers overnight, it was found that a fan-shaped spore print

[1] Received for publication Feb. 11, 1924.
[2] Reference is made by number (italic) to "Literature cited," p. 756

had been formed, spreading away from the mouth of the cup (fig. 1, A, B). These spores, mounted in water, appeared not to differ from ordinary aecidiospores, but examined dry directly on the slide on which they had been discharged, it was apparent that some of the spores had small spherical bodies attached to their walls. Many such granules lying scattered about over the field covered were at first thought to be particles of dust, but on going back to aecidia on fresh leaves and looking down into the aecidium cups it was found that the spores showed these bodies on their surfaces before being discharged. By carefully dislodging the bodies from the spores in dry mounts it became evident, as it had not been in liquid mounts, that some of them were located directly over the germ pores and that probably each germ pore originally was capped by one of these globules. The part that they might play in spore discharge became clear from a study of their development.

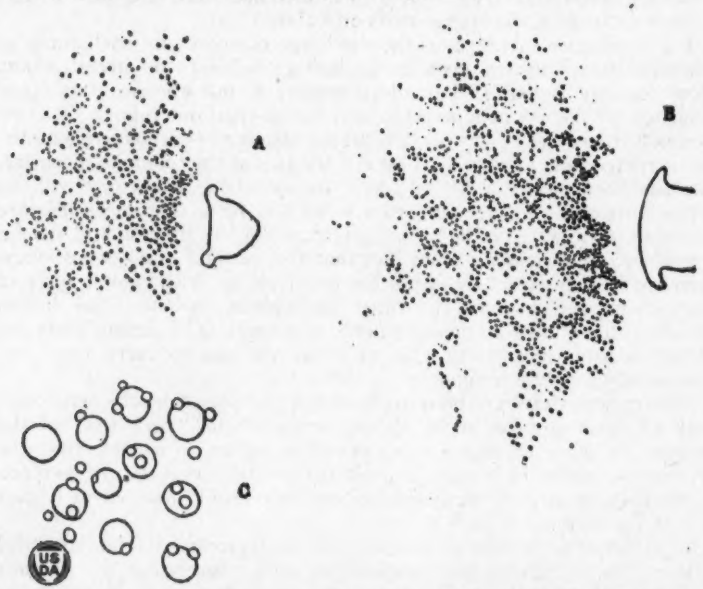

FIG. 1.—*Gymnosporangium myricatum*. A, B: Spore prints formed after dissecting out an aecidium, and laying it down on a glass slide in a damp chamber. The print was photographed and the diagram made from the photograph. Some of the spores were shot out distances equal to about 100 times the diameter of the spore. They might have been thrown farther if the aecidium had been placed somewhat above the glass support. C: Outline of spores showing comparative sizes and numbers of plugs that still remained attached to the spores after their flight.

Material for a cytological study of the bayberry rust was obtained by cutting out very small pieces of leaf or stem bearing young aecidia and fixing them in Flemming's fluid. The triple stain differentiates very well the structures with which we are particularly concerned.

Between the lowest spore cell being cut off from the basal cell and the outermost spore which is fully mature and is about to be discharged, there exists a number of spores in all stages of development (fig. 2). These spore chains are clearly prismatic in cross-section because they are under strong pressure, not only from the sides but from below as well, and the individual spores and intercalary cells are more or less angular in outline. Spores in adjacent chains are cut off at different

levels so that no two spores have exactly the same shape as they exist under these pressures. Only one layer can be made out in the wall of the youngest spore in the chain. Beginning with about the fourth spore from the base, differentiation of the wall into three distinct layers can be observed. The outer and inner layers are very thin, while the middle layer is somewhat thickened. Pore formation begins at about this stage. All of the spore wall takes a deep orange G stain except that region where the pore will later be found. This part becomes considerably thickened, remains slightly separated from the rest of the "exospore" and takes a brilliant gentian violet stain; the older the spore the larger and more easily distinguished becomes the violet-stained portion, which for convenience may be referred to as a "plug." It is very much flattened (fig. 3); its thickness is only about half its diameter. Viewed edgewise it appears to be made up of striae similar to the lines in the walls of the peridial cells. At least it is not homogeneous in its structure. Viewed flatwise it seems to be composed of wedge-shaped segments placed edge to edge. The prevailing shape of the fully formed plug is that of a basin or a hatter's block (fig. 3, B). The writer's conception of the mechanics of spore discharge follows.

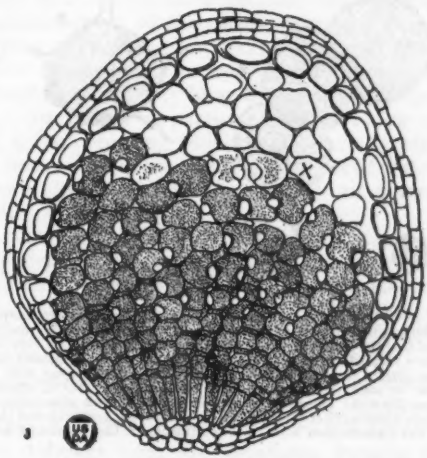

FIG. 2.—*G. myricatum*. Section of young aecidium. (See text for further explanation.)

The aecidium is slightly contracted where it comes in contact with the lower epidermis of the leaf, or if not, the effect is the same because the epidermis offers more resistance to the forces of expansion developed by the growing sorus than do the cells of the mesophyll. The spore walls are highly elastic so that at the spot opposite each plug the adjacent wall is much indented (fig. 3, A). As the maturing spores are being pushed upward new adjustments are brought about as the spores slide by one another over the separating plugs. While the shape of a spore under pressure is very irregular, it loses its angularity as soon as it is forced past the level of the epidermis so that these confining pressures are removed. The parts indented by the plugs spring back into place. The reaction of the walls of other spores touching this one is such as to violently expel the spore from the cup.

A section of a young aecidium is shown in figure 2, the layers of cells surrounding the aecidium being shown diagrammatically. The outlines of peridial cells, spores, and pore plugs were traced with the aid of a camera lucida. The unshaded spores in the upper part of the aecidium have no cytoplasmic contents. They are degenerating and by so doing may supply mucilaginous substances which by swelling assist in rupturing the

epidermis. The shading in this figure is simply to indicate cytoplasmic contents. The open spaces shown in this section are clearly due to shrinkage in fixation. If the aecidium were open and the degenerated spores cleared away, the spore just below X would be in a position to be shot out with considerable force.

Every spore is at first interlocked with its neighbors through the plugs indenting their walls and because the spore walls are rough or finely warted. At first the system only gradually moves out of the state of equilibrium. New adjustments are continually taking place until one spore is forced into the critical position necessary before it can be set free.

The correctness of the above explanation of the mechanism by means of which the aecidiospores of *G. myricatum* are ejected from the sori with such velocity can be demonstrated by the use of tennis balls and marbles. Confine the balls under pressure from all sides, except from

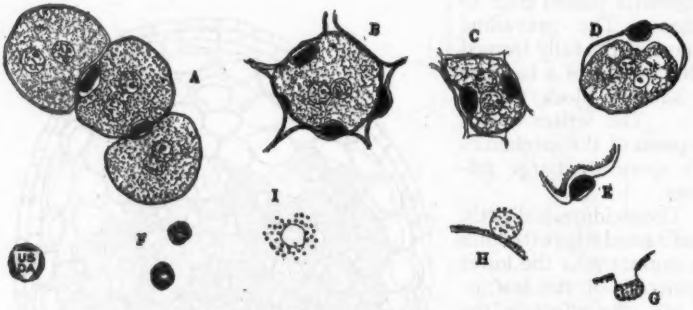

FIG. 3.—*G. myricatum*. A: Section of three adjacent spores showing pore plugs as they lie in the spaces which will become the germ pores. Note that the wall of the adjacent spore is indented in each case, and that the plug is covered on the outer and inner sides by very thin membranes. B: Section of a young spore parallel to the surface of the leaf. Two pore plugs and the edge of a third show in the section; one plug in an adjacent spore. C: Section of young spore with two large plugs. The indentations are due to side pressures and not to shrinkage. D, E show shrinkage of spore contents away from the wall. Material preserved in alcohol several years before imbedding. F: Surface view of pore plugs, showing that these bodies do not have a homogeneous structure. G, H: Position of pore plugs in dry mounts from herbarium specimens. I: Germ spore as it appears after dislodging the pore plugs

above, with marbles thrust in between them where they touch. By gradually increasing the pressure from the sides the balls become more and more compressed but remain in position. Now if pressure is applied to one of the balls from below it gradually moves up until the resultant of forces is such as to overcome the friction between the balls and between them and the marbles, and the ball will be liberated and suddenly shot upward. If the convexed bottom of a well chosen tin pan is pressed inward it will remain concave until set in motion by a slight pressure, or by warming the pan. The bottom moves out slowly at first until the critical position is reached, when it will suddenly complete the return with much noise. By placing two such pans together, bottom to bottom, one will be thrown upward violently, especially if the two bottoms in contact suddenly become convexed at the same time. The conidium of *Empusa grylli* is thrown off violently on the same principle. The two elastic membranes between the spore and its support suddenly become convexed. In this case the little columella may in reality function the same as the pore plug does in the Myrica rust, namely, as a protuberance against which the elastic membrane of the spore can react all the more

strongly. Similarly, a tennis ball compressed over a marble on a table will be thrown farther upward when the confining pressure is suddenly removed than it will be if compressed against the table alone with the same force, and then released.

Very little work has been done on aecidiospore discharge, so that it is impossible to explain the necesssity for any special device for insuring that the spores be ejected forcibly from the aecidium as soon as they mature. The aecidiospores of a great number of rusts are provided with germ pores and it is not unlikely that in some of the species the spores are thrown out with considerable velocity. It is conceivable that in some cases this would be of decided advantage to the rust. In the rust on Myrica most of the aecidia develop on the under side of the leaves (*5*). If the spores dropped out of the cup as soon as mature, they could be borne away by the wind just as well as if they had been shot out a short distance. On the other hand, these spores are rather short-lived and as they are somewhat waxy they would tend to cling or mass together in the cup and fall out in clumps as do the spores of the short-cycled orange-rust of Rubus (*4*). The aecidiospores of this blackberry rust develop sporidia on promycelia as they germinate, functioning as teleutospores. It is an unreported fact that these spores frequently germinate during wet weather or on warm dewy nights as they hang together in masses on the under side of the leaves. The spores are particularly fitted to function in this way by developing a waxy coating which holds them together. Sporidia developed on the under side of the leaves a few feet from the ground would be much more likely to be borne away by air currents than would the heavy aecidiospores themselves.

The aecidiospores from roesteliae of other Gymnosporangia are certainly not discharged with violence. The writer's attention has not been attracted to any such persistent plugs developed in pore formation in roesteliae examined. In *G. clavariaeforme* where the germ pores are large and distinct from the first, the method of pore formation approaches that of pollen grains of geranium as described by Strasburger. In place of a thickened lid covering the pore, a granular substance is extruded which at first has the shape of a plug. It takes the orange G faintly and connects with the spore contents. The outer portion of the extruded mass is certainly not very different from the substance forming the thickenings on the exospore, but as the spore approaches maturity it is reabsorbed or withdrawn. The aecidiospores of this species, which are comparatively long-lived, collect in large quantities in the cancellate peridia and are allowed to escape through the hygroscopic action of the peridial cells.

A beautiful example of modification of the coating of the exospore to better adapt the aecidiospore for proper distribution is found in connection with the orange-rusts on blackberry. There can be no question that the two orange-rusts are very closely related. The preponderance of evidence is that the short-cycled rust has been or is being derived from the long-cycled form. The aecidiospores of the latter should be distributed widely so as to bring about numbers of sporophytic infections in new regions. This large and irregular Gymnoconia aecidium is of the caeoma type and as the spores are not provided with germ pore plugs it is difficult to imagine how the spores can be so confined under pressure as to be discharged at maturity in any way comparable to the method worked out in the case of the bayberry rust. Nevertheless when we examine blackberry leaves bearing the long-cycled orange-rust, we find that these aecidiospores

are also being discharged with considerable violence. If a Petri dish containing a thin layer of agar is inverted and a leaf bearing aecidia is placed in the cover so that the surface of the leaf which is covered with aecidia faces upward, it can be proved that the spores are frequently thrown vertically to a distance of 4 or 5 mm. or more. Many spores not hitting the agar, or if so, not sticking, fall back on the leaf or on the glass at some distance. Further study of the mechanics of spore discharge by the orange-rust will certainly prove interesting. These spores are not very waxy and tend to fall out of the sori soon after maturity, and they can stand a certain amount of drying. There is, however, enough wax on the exospore to insure that many of the spores as they fall will stick to the other leaves of the plant on which they were originally developed. By this compromise we find that teleutospores are commonly developed on the original host plant as well as on plants some distance away. The waxy coating is more highly developed on the aecidiospores of the short-cycled orange-rust, and as noted previously, this tends to hold the spores together in the sorus on the leaves well above the ground. Germinating in this position the resulting sporidia, well adapted for dispersal by air currents, begin their flight from an elevated position. The teleutospores of *Puccinia malvacearum* germinate in the sori as they hang on the living leaves, as soon as they are mature.

If one desires to infect a root shoot of a blackberry with the short-cycled orange-rust, he will be more successful if he sows sporidia on the shoot. If he sows aecidiospores he will be less successful; one reason for this may be that the sporidia which are to produce the infection are discharged from the promycelia with sufficient force to carry them away from the very place where they should come to rest if infection is to be obtained.

Not all of the aecidia of the bayberry rust are borne on the under side of the leaves. In many cases the ends of young branches and the fruit buds are covered with them so that the cups open out in all directions. This is especially true of the rust on the "sweet fern," Comptonia, where the burlike fruits and entire terminal parts of certain branches are attacked. The leaves bearing aecidia are also contorted and coiled into a form resembling that of a ram's horn (*4*). Such locations bring the aecidia into positions unfavorable for spore discharge unless the spores are started off with an initial impulse. Without entering into an extensive discussion of the formation of germ pores in other types of spores, it may be interesting to note that Strasburger and others have studied pore formation in the walls of pollen grains of several species of plants, and further that there are homologies and analogies between the pore plugs described above and germ pore lids in certain pollen grains.

Strasburger (*8*) in his well-known work on the structure and growth of cell membranes describes the development of the walls and germ pores of several different kinds of pollen. Two layers are differentiated; the outer layer is referred to as the exinium, or exine, which is commonly called the exospore. An inner layer Strasburger identifies by the terms intinium or intine (endospore). In the geranium (*8*) (fig. 27–38), thickening of the outer wall of the pollen grain does not occur at certain points. These thin places in the wall are the germ pores. Due to pressure from within, some of the cytoplasmic contents of the cell along with some of the endospore, are forced through the germ pores and form papillae. The granular contents become lined up in striae and stain

brown at first, then blue, with iodin. While in this condition the papillae superficially resemble very closely the striated germ pore plugs of the Myrica rust being considered here; they are of an entirely different origin and not to be compared, except as to location, with respect to the germ pores. The lids or caps which cover the germ pores in Cucurbita pollen (Strasburger (*8*), fig. 77–84) are more nearly analogous because the lids are developed out of the exospore layer. Had the caps developed beneath and not out of the original cell wall, they could be said to be homologous to the exospore thickenings of aecidiospores and of zygospores of the Mucoraceae.

Vuillemin (*9*) who studied the development of the walls of zygospores of Sporodinia and other Mucoraceae, recognizes five different layers or membranes. The innermost layer is composed of a granular matrix. The next part of the wall is referred to as the cartilaginous layer. This is bordered by a middle, thin membrane. The fourth layer is usually heavily carbonized and developed into characteristic warts or spines. Surrounding the young zygospore is the thin membrane, originally the primitive wall of the progametes.

Dangeard (*2*) features the two membranes which are easily separated. The exospore, warted and carbonized, originates as a separate cone-shaped thickening beneath the thin outer membrane. The endospore is the thick layer within which follows the undulations of the exospore. Lendner (*6*), Moreau (*7*), and others studying zygospore formation also show that in several species the warts or thickenings originate as separate cone or dome shaped structures in a matrix and by lateral extention fuse together to form the firm carbonized layer of the spore wall. Without lies the primitive membrane of the gametangia; within there remains a separable membrane of considerable thickness in contact with the cytoplasmic contents of the zygospore. Disregarding the outer thin layer of the young spore wall which is usually not to be distinguished in the mature spore, the two remaining layers are referred to as "exospore" and "endospore".

The pore plugs in the aecidiospore of the bayberry rust develop in the matrix or layer out of which the thick warty or echinulate exospore is formed but outside of this heavy wall, what the writer has interpreted as the thin primitive membrane of the spore wall can be seen if the spore cells are plasmolized. The inner membrane then also becomes visible.

As noted above, Dietel has shown that sporidia from certain teleutospores are sometimes projected 0.6 to 0.8 mm. The writer has made no attempt to learn just how far a spore can be thrown as it is discharged from the aecidium. The spore print (fig. 1) shows that every spore is forcibly expelled; there is always a clear space around the mouth of the cup where no spores have fallen. Not many spores are projected a distance of over one hundred times their diameter when an aecidium is laid directly on a glass. As the diameter of an aecidium is only a few times that of a spore, it can be seen that a spore has not long to fall before it hits the glass. Spores shot from aecidia growing horizontally on buds or coiled leaves would certainly be scattered much farther and more efficiently if they were given an initial horizontal velocity.

Germ pores of certain teleutospores are very commonly figured as being covered by very delicately bounded papillae. Structures like the papillae in the geranium pollen represent in part extruded cell sap os cell contents. The pore plugs are persistent morphological structurer

which often cling to the spores very firmly. Such bodies can be found on the aecidiospores of the Myrica rust in any good herbarium specimen. Regardless of the actual mechanics of the discharge in this case, the spore in its flight no doubt follows closely the sporabolic curve described by Buller for the Basidiomycetes, so that beyond a certain and definite limit it probably makes little difference so far as the length of its horizontal flight is concerned, how high above the glass slide the aecidium is supported in the experiment referred to. The spore is shot off with a very great horizontal velocity which carries it almost straight out for a certain distance. Because of air resistance it then takes an abrupt turn and falls straight down unless carried away by currents.

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HASTENING THE COLORATION OF LEMONS[1]

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INTRODUCTION

Lemons are picked not only according to color, but also according to size. Thus all fruits that have become yellow on the tree are picked, and in addition those with a green color if they have reached a certain size as determined by a wire ring carried by the picker. As a result, when lemons reach the packing house a proportion, which varies with the field and with the season, is green in color. Such fruits are sorted into light green and dark green grades, and the yellow color is subsequently brought out by one of two general methods, the choice of method depending upon market conditions, that is, the time at which the house manager wishes to have the fruit ready for shipment.

If a delay in shipment is desirable, the green lemons are placed in storerooms, usually in a basement, at a temperature of from 50° to 55° F., with a humidity of about 80 per cent. The fruit becomes yellow in from 30 to 60 days.

If, however, the demand for fruit is brisk, the "forced coloring" method is used. This system has been much improved since its introduction many years ago. According to the original procedure, the fruit is placed in rooms or tents heated with kerosene stoves, with the result that the lemons turn yellow in one or two weeks. A source of humidity is often, although not always, provided to prevent shrinkage.

It was thought that the coloration was brought about by the temperature and humidity conditions in the heated rooms, but Sievers and True (*17*)[2] conclusively showed that the results were produced mainly by the gaseous combustion products from the kerosene stoves. Their experiments showed further that the gases did not lose their effectiveness by being conveyed from one room to another by pipes, using either forced or natural draft. Hence the more general method now in use consists in generating the kerosene-stove combustion products, hereafter referred to as "stove gas," in a separate building, called a generator room, and carrying the gases through conduits to the various fruit rooms.

Answers to a questionnaire sent to packing-house managers showed a general lack of agreement on most of the important details of operation. Uniformity in color and quality of fruit was not obtained, and it seemed impossible to fix upon any standardized procedure as being the one that gave the best results. This condition probably was due to the fact that no one knew what gas or gases in the sweat-room atmosphere caused the change. Since this seemed to be the critical question, experiments were started to determine if possible the identity of the gaseous constituent responsible for the coloration of the fruit.

[1] Received for publication, Jan. 23, 1924.

[2] Reference is made by number (italic) to "Literature cited," p. 768–769.

EFFECT OF GASES ON COLORATION OF LEMONS AS DETECTED BY STANDARD ANALYTICAL METHODS

STOVE GAS

No analyses of the combustion products from kerosene stoves were found in the literature. Analysis of samples taken from the air above the burning flame showed: Carbon dioxid (CO_2), 0.73 per cent, 0.63 per cent; oxygen (O_2), 20.01 per cent, 19.94 per cent; no unsaturated gases; and carbon monoxid (CO), 0.48 per cent, 0.05 per cent. Analysis of samples of ordinary air, using the same apparatus and reagents, gave: Carbon dioxid (CO_2), 0.0 per cent, 0.04 per cent; oxygen (O_2), 21.04 per cent, 21.11 per cent; no unsaturated gases; and no carbon monoxid (CO). The oxygen percentages were, of course, too high, but no attempt was made to determine the cause. The data indicated that it was not feasible to search for the effective gas by ordinary gas-analytical methods.

AUTOMOBILE EXHAUST GAS

Sievers and True (*17*) pointed out that the exhaust gas from automobile engines can color lemons. The composition of this gas has been very carefully determined (*8*, *2*). Emphasis was laid upon completeness of combustion, however, because of its importance from the standpoint of fuel economy, and upon the quantity of carbon monoxid formed, because of its toxicity to man. The other gases present were not sorted out individually but were listed as heavy hydrocarbons, unsaturated gases, illuminants (C_nH_{2n}), etc.

SWEAT-ROOM ATMOSPHERE

S. A. Weirman, formerly of the laboratory of fruit and vegetable chemistry, United States Department of Agriculture, analyzed many samples of air taken from various sweat rooms in which fruit was being colored by kerosene stove gas. The quantities of carbon dioxid varied from 0.1 per cent to about 1.5 per cent, in one case reaching 4.9 per cent. The oxygen content was generally about 18.6 per cent. Carbon monoxid in small quantities was found in some samples. Less than one-third of the samples showed the presence of heavy hydrocarbons, the greatest quantity being 0.25 per cent.

CARBON MONOXID AND CARBON DIOXID

The two gases among the combustion products positively identified by these methods are carbon monoxid and carbon dioxid. Carbon dioxid in a wide range of concentration was tried by Weirman, but it failed to produce the desired result. He found also that carbon monoxid in high concentration did not induce coloring. Later experiments, however, have shown that weaker concentrations are effective. Eight per cent of carbon monoxid caused coloration in about one week, even with gas that had been bubbled through bromin water to remove unsaturated hydrocarbons. However, 1 per cent of carbon monoxid was ineffective, only a slight change resulting, and one part of carbon monoxid in 1,000 parts of air gave no detectable change.

Therefore carbon monoxid is not the effective constituent, since concentrations of this gas sufficient to produce coloring would cause the death of

human beings in a few minutes. Such concentrations do not exist in commercial sweat rooms.

EFFECT OF GASES DETECTED BY SPECIAL METHODS

A stream of stove gas was bubbled through Pettenkofer tubes, some filled with 10 per cent potassium hydroxid solution and some with distilled water. After about three days the solutions were poured out and the liquids were tested for various substances.

A portion of the aqueous solution obtained in this way was tested for formaldehyde by the Rimini test (*15, p. 85*). The result was positive. Indeed, formaldehyde caused lemons to turn yellow, but the color was too brassy, and sunken spots formed in the rind wherever drops of vapor condensed. A similar result was obtained with acetaldehyde.

The solution of potassium hydroxid through which the stove gas had bubbled was tested for nitric oxid by the method described by Dennis (*5, p. 218*). A distinctly positive test was given. Nitric oxid, generated from sheet copper, potassium nitrate, and concentrated sulphuric acid were applied to green lemons in desiccators. None of the lemons turned yellow, but some of them showed surface injuries.

The water and potassium hydroxid absorptions were also tested for the presence of phenols by Scott's method (*16*). A positive test was obtained. The vapor from phenol turned green lemons yellow in about six days and no blemishes were caused. However, it did not seem possible to make any practical application of this fact.

Stove gas was bubbled through Pettenkofer tubes containing solutions of ammoniacal and of neutral silver nitrate. A small quantity of a precipitate, probably silver acetylide, was formed. On adding acid, this decomposed, giving off a gas with an odor of acetylene. Acetylene from a commercial cylinder at a concentration of 1 to 1,000 was effective in bringing about coloration. Since commercial acetylene is said to contain traces of other gases, however, acetylene of greater purity was prepared by treating ethylene dibromid with alcoholic potash (*14, p. 84*). The gas generated in this way caused no change in the coloration of lemons after exposure for seven days to concentrations of 1 to 100, 1 to 1,000, and 1 to 50,000. Hence the coloration obtained by the use of commercial acetylene is thought to have been caused by the impurities present and not by the acetylene itself.

REMOVAL OF EFFECTIVE CONSTITUENT FROM STOVE GAS BY ABSORBENTS

Since the effective constituent seemed to be present in the stove gas in low concentrations only, an effort was made to absorb this trace by reagents. In the apparatus devised for this work (fig. 1), a current of gas from the stove was drawn through the system by reduced pressure at *S*, the reduction being equivalent to about 7 inches of water below atmospheric. By a stopcock at *C*, bubbles from 3 to 4 mm. in diameter were made to follow one another in rapid succession up the tube in which they were washed by the absorbing liquid. From 7 to 10 seconds were required for a bubble to travel the length of the tube.

It can not be stated that complete absorption took place, since, for the complete removal of a gas by a liquid, shaking for several minutes is often recommended. When solid-absorbing reagents were used

they were placed in straight glass tubes, 4 to 5 feet long and 3/4 to 1 inch in diameter. If necessary, gas-washing bottles were inserted at *WB*, the first filled with the proper liquid to remove any vapors carried over the absorption tubes and the second to keep the gas saturated with water vapor. The residual gas then passed into the desiccators containing green lemons. In order to permit a conclusion as to the effect of the absorbing substances, two control lots were provided. One was a similar lot of fruit treated with a stream of unabsorbed stove gas, and the other was a desiccator of green fruit aerated each day with outdoor air, receiving no gaseous treatment.

The following solid reagents did not remove the effective constituents from the stove gas: Granular calcium chlorid, soda lime, activated charcoal, and silica gel. Complete removal, however, was obtained by means of hopcalite (*9*, *p. 110*). Stove gas dried by calcium chlorid and passed through a small tube containing about 25 gm. of granular hopcalite was no longer capable of inducing coloration of green lemons. This reagent is noted for its ability to oxidize carbon monoxid, but the firm manufacturing it states that not only carbon monoxid but many other oxidizable gases are acted upon by it.

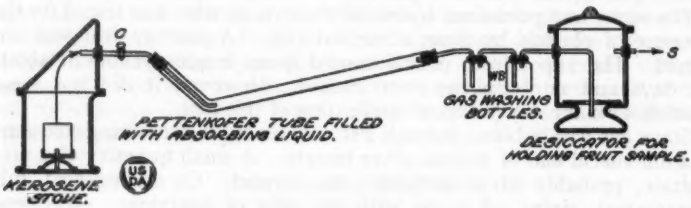

Fig. 1—Apparatus for removal of constituents from stove gas.

The following liquid-absorbing reagents did not completely remove the effective constituent from stove gas: Water, concentrated sulphuric acid, 50 per cent potassium hydroxid, 0.75 per cent, potassium permanganate, 10 per cent silver nitrate, and saturated aqueous solution of mercuric acetate.

When the stove gas was bubbled through saturated bromin water and when the bromin vapor was removed by dilute sodium hydroxid, however, the residual gas did not color green lemons. This result suggested the desirability of testing the effect of unsaturated gases such as ethylene.

PRELIMINARY LABORATORY EXPERIMENTS WITH ETHYLENE IN LOW CONCENTRATIONS

In the preliminary experiments ethylene was generated by dropping ethyl alcohol upon phosphoric acid as described by Doubt (*6*). When it was found that ethylene could be bought as a compressed gas in steel cylinders, most of the experiments were conducted with gas from this source. An analysis of gas from the cylinder showed that 97.7 per cent by volume was absorbed by bromin water.

For testing the effect upon green fruit of ethylene at low concentrations, gas from the cylinder was diluted with air to make ethylene-air mixtures of 5 per cent, 1 per cent, 0.1 per cent, 0.05 per cent, and 0.01 per cent. Green fruits, the number varying from 6 to 24, were placed in

large glass or metal vessels with a capacity of from 2,500 to 50,000 cc. Allowance being made for the volume occupied by the fruit, gas from the stock ethylene-air mixtures was added from a gas burette in sufficient quantities (usually 10 to 50 cc.) to give the desired concentration of ethylene in the atmosphere surrounding the fruit. This was done once or twice daily. The vessels containing the fruit were aerated by outdoor air for purposes of ventilation before the gas was added. Control lots consisting of fruit receiving treatment with outdoor air only were provided in each experiment. The following proportions by volume of ethylene to air were tried: 1 to 1,000; 1 to 5,000; 1 to 10,000; 1 to 50,000; 1 to 100,000; 1 to 200,000; 1 to 250,000; 1 to 500,000; 1 to 1,000,000; 1 to 2,000,000; and 1 to 5,000,000.

In all cases coloration of green lemons resulted. The effect was not in proportion to the concentration. Concentrations from 1 to 1,000 to 1 to 200,000 produced approximately the same effects, coloring the lemons in from 5 to 8 days. Concentrations down to 1 to 2,000,000 were somewhat less effective, requiring 1 or 2 days longer. Concentrations of 1 to 5,000,000 required about 14 days, indicating that this concentration represents the highest dilution at which coloration is markedly hastened.

The effect of ethylene was further checked in one case by bubbling the gas-air mixture through bromin water before permitting it to come in contact with the fruit. In this case coloration did not result.

EXPERIMENTS WITH ETHYLENE UNDER COMMERCIAL CONDITIONS

EFFECTS OF ETHYLENE AND OF STOVE GAS

Forty-eight boxes of dark-green lemons, divided into two equal lots, were placed in two large sweat rooms of commercial size (about 6,600 cubic feet), provided by a California citrus association. In one room a kerosene stove was burned throughout the experiment in a manner identical with present commercial practice. Into the other ethylene was measured out from the cylinder through a gas meter. Twenty-five applications of the gas were made at intervals of six hours, a total of 48.55 cubic feet being liberated into the room. At first the air was stirred with an electric fan to distribute the ethylene, but later this was discontinued, being unnecessary. The temperatures and humidities of the two rooms were kept the same by close attention and hand regulation, heat equal to that formed by the kerosene stove being provided for the second room by means of hot-water coils near the walls of the room. The temperature ranged from 60° to 65° F., and the humidity from 80 to 90 per cent. The fruit in both rooms colored in eight days and no difference in the two lots could be noted, either with respect to the color developed or to the commercial quality of the finished product.

METHOD OF MEASURING AND APPLYING THE GAS

It was neither convenient nor accurate to measure the gas from the cylinder with a gas meter, but the apparatus shown in figure 2 was satisfactory in every respect for this purpose. *A* is the cylinder of compressed ethylene, and *B* is an empty cylinder capable of withstandin pressures up to 50 pounds. *B* serves as a "measuring cylinder." *C* is a pressure gauge, reading in pounds per square inch. It is desirable to

place a pet cock between the gauge and the measuring tank in order to protect the gauge from the sudden increase in pressure when the gas is released from the ethylene cylinder. *D* is the rubber hose outlet. To measure gas with the apparatus, valve *E* is closed and valve *F* is opened. As ethylene is forced over into *B*, the reading on the pressure gauge rises. If *F* is now closed and *E* opened, gas is released from the cylinder and the pressure-gauge reading falls. After a few such operations *B*, of course, becomes filled with pure ethylene gas at atmospheric pressure. Gauge readings were calibrated by collecting and measuring the released gas. In this particular case a reading of 10 pounds was equivalent to 1 cubic foot of gas. The measuring apparatus was mounted on a wheel truck for convenience in gassing the various rooms or experimental lots of fruit.

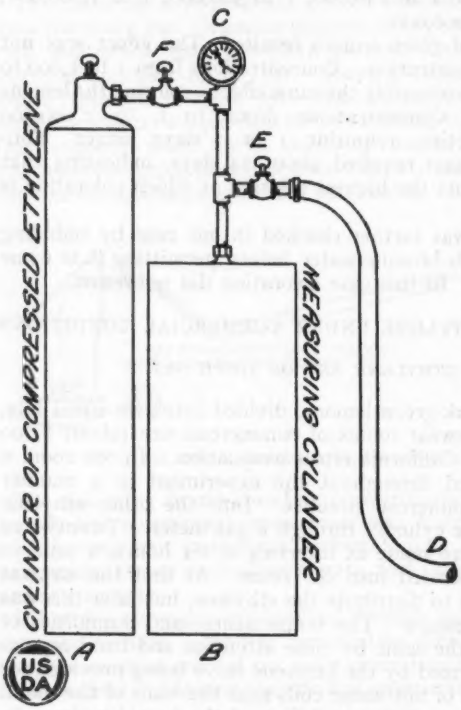

Fig. 2.—Apparatus for measuring ethylene from cylinder.

EFFECT OF TEMPERATURE ON RATE OF COLORATION

Three of the large rooms previously mentioned were used in this experiment, the first being kept at 78° to 83° F. (average, 82° F.), the second at 64° to 69° F. (average, 68° F.), and the third at 52° to 60° F. (average, 57° F.). Heat was furnished by an automatic hot-water heater in the hallway in the basement. The hot water circulated in pipes attached to the side walls of the rooms and the temperature was regulated by a valve in the hot-water pipe line. Moisture for humidifying the rooms was produced by means of large atomizers operated by compressed air, which gave a very fine mist. Seventy-two boxes of green lemons, divided into three equal lots, were placed in the rooms. Every six hours 1 cubic foot of ethylene from the cylinder was measured out into each room.

The fruit in the room at 82° F. colored up in 4 days, that at 68° F. in 5 days, and that at 57° F. in 9½ days. In all lots, the color was good. In the room at 57° F. the buttons were firmer and less discolored than those of the fruit in the other rooms.

The effect of higher and lower temperature was tested in the laboratory with small quantities of fruit. At a temperature of 45° F. and with ethylene of a 1 to 1,000 concentration, no development of color took

place in 10 days. In an incubator at 93° F. and with ethylene of a 1 to 10,000 concentration, coloration took place in 9 days, but the color was not as well developed as with a similar lot of fruit held at a temperature of 68° to 72° F. Thus a low temperature practically inhibited coloration and a high temperature reduced the rate.

EFFECT OF REDUCING THE NUMBER OF GAS APPLICATIONS

In the first experiments in commercial rooms, gas applications were made four times a day. More than two a day would be inconvenient in practical work. Accordingly, about half a carload of green lemons, representing boxes of fruit from several groves, were placed in a sweat room and one cubic foot of ethylene was released into the room twice daily. For the purpose of ventilating the fruit, once daily, just before a gas application, the ventilator and doors were opened for one hour, and air was drawn into the room by means of an electric fan. The temperature of the room during the experiment was 68° to 73° F. (average, 70°). The humidity was 75 to 90 per cent (average, 81 per cent).

The different lots of fruit in the room colored at different rates. The first lot was removed at the end of 4 days, and other lots at the end of 6, 7, and 8 days. One lot was not completely colored at the end of 10 days. The color and condition of the fruit at the end of the experiment were satisfactory. It is not known what concentrations of ethylene existed in the room during the experimental period, since no method of estimating ethylene in such low concentrations was found. After liberation of the gas diffusion losses at once began. The extent of these losses could not be determined, but data in an unsigned article (*1*) regarding air exchange in a closed room indicate that they would be large. The experiment, however, showed that two applications of gas a day were sufficient to induce coloring.

COLORING FRUIT IN TENTS

Fruit was not placed in a special room, but the boxes were stacked in an open hallway and covered with two thicknesses of ordinary cotton tent canvas, such as is used in fumigating with hydrocyanic acid gas. Four times daily the outlet hose from the cylinder was pushed under the edge of the tent and ethylene was admitted to make a concentration of ethylene amounting to 1 part in 250. This lot was exposed to the temperature of the outdoor air and varied from 50° to 76° F., with an average of 58°.

At the end of 10 days the fruit was full yellow. The color was slightly paler and more attractive than that of similar fruit colored in regular sweat rooms. It appeared that the tent covering retained the gas well enough to permit coloring to proceed in a satisfactory manner.

CONDITIONS PREVENTING OR RETARDING RATE OF COLORING

HIGH CONCENTRATIONS OF ETHYLENE

Large bottles were filled with a gas mixture consisting of 80 per cent ethylene and 20 per cent oxygen by volume. By water displacement, a supply of gas was pushed over once each day into desiccators containing green lemons. For comparison, a second lot of fruit was treated in a similar way, except that the ethylene concentration was 1 to 1,000. A third lot received no gaseous treatment but was aerated with outdoor air each day. At the end of 7 days, the lemons in the third (control)

lot were still green, those in the second lot (ethylene 1 to 1,000) were yellow, and those in the first lot (ethylene 80 per cent) were slightly more yellow than the controls but not more than one-eighth colored, the buttons being firm on all but one. High concentrations of ethylene appear to retard the rate of coloring.

LACK OF OXYGEN

Pure nitrogen, generated from ammonium chlorid and sodium nitrite, was collected over water in a 19-liter bottle. To this nitrogen ethylene was added to make a concentration of 0.5 per cent by volume. By water displacement this gaseous mixture was pushed over into a desiccator containing green lemons. In order to remove the oxygen as completely as possible, the bottom of the desiccator was covered with alkaline pyrogallate, which was also placed in wash bottles through which the gas was bubbled before entering the desiccator. For comparison, another lot of fruit was treated in an exactly similar manner except that outdoor air was used instead of nitrogen and water was substituted for alkaline pyrogallate. Fresh quantities of gas were pushed over into the desiccators each day. The control lot was colored yellow at the end of eight days but the lot receiving no oxygen remained green. A duplicate experiment, using 1 per cent of ethylene, gave similar results. Oxygen appears to be necessary for coloring.

EFFECT OF ETHYLENE AND OF STOVE GAS UPON RESPIRATION OF LEMONS

The experiments showing that coloration was prevented or interfered with by high and low temperatures and by high concentrations of ethylene, that it was favored by intermediate temperatures, and required oxygen, indicated that coloring was hastened by conditions that were favorable to the life processes of the fruit. Furthermore, whenever ethylene or stove gas was used, coloration was accompanied by the loss or loosening of the buttons (calyx and a portion of the clipped fruit stem). The cells at the absciss layer were greatly increased in size, that is, growth and enlargement had taken place. In many cases there was an extrusion of tissue just below the buttons, a condition similar to that described by Doubt (6) and others. If ethylene and stove gas induce coloration by a stimulation of the growth of the cells, or by increasing their life activity, the respiration of the treated fruit should be increased.

Ethylene in concentrations of 1 to 1,000 and 1 to 1,000,000 markedly increased the carbon dioxid output, the percentage increases ranging from 100 per cent to about 250 per cent.[3] A comparison was made of the effect of ethylene and of stove gas upon the respiration of lemons. Six lemons, placed in each of six desiccators, received the following treatment:

Lots A and B.—A current of stove gas was drawn through the desiccators for six hours each day. The desiccators were then closed and placed in an incubator at 25° C. until half past 8 o'clock the following morning.

Lot C.—Ethylene to make a concentration of 1 to 10,000 was added twice each day, after thorough aeration in outdoor air. The desiccator was then placed in the incubator simultaneously with lots A and B.

[3] DENNY, F. E. THE EFFECT OF ETHYLENE UPON THE RESPIRATION OF LEMONS. *In* Bot. Gaz. Not yet published.

Lot D.—Same treatment as lot C, except that the concentration of ethylene was 1 to 200,000.

Lots E and F.—These control lots received no gaseous treatment but in other respects were handled in the same way as lots C and D.

Every other day the fruit was removed from the desiccators and aerated for one-half hour. The air in the desiccators was forced out by filling them with water, the fruit was replaced, and a current of air was aspirated through the system for 1½ hours, the carbon dioxid being absorbed in barium hydroxid solution. From the data thus obtained the number of milligrams of carbon dioxid per kilogram per hour was calculated. The results are shown in Table I and graphically in figure 3.

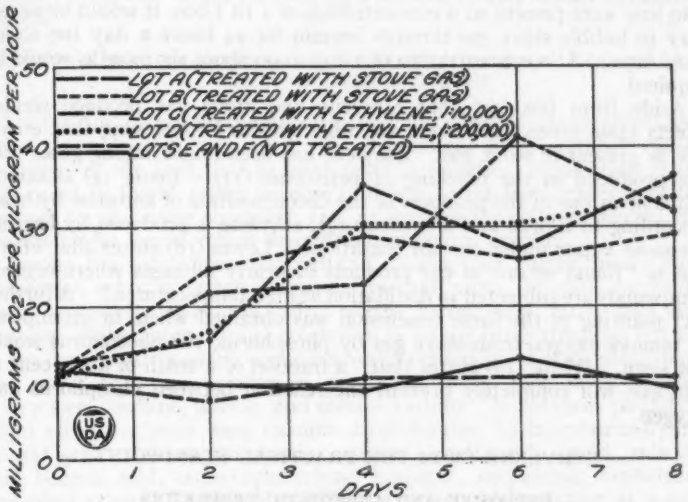

FIG. 3.—Effect of stove gas and of ethylene on respiration of lemons.

The respiration was increased by both ethylene and stove gas, the percentage increase being from 150 to 250 per cent. No essential difference between ethylene and stove gas with respect to the increase in respiration was shown. Further experiments are needed to decide this point.

TABLE I.—*Effect of ethylene and of stove gas upon respiration of lemons*

Lot No.	Weight of fresh fruit.	Treatment.	Rate of respiration.				
			Start.	Second day.	Fourth day.	Sixth day.	Eighth day.
	Gm.		*Milligrams of carbon dioxid per kilogram per hour.*				
A	707	Stove gas.....	12.3	15.1	34.9	26.4	35.8
B	699	.....do.....	11.2	24.3	28.6	24.8	25.8
C	698	Ethylene, 1 to 10,000....	10.5	21.0	22.0	41.1	30.6
D	649	Ethylene, 1 to 200,000...	11.3	15.4	29.8	28.8	32.9
E	658	Control.....	10.1	7.1	10.1	10.1	10.1
F	693	.....do.....	11.5	9.1	8.7	12.5	9.6

IS ETHYLENE PRESENT IN THE COMBUSTION PRODUCTS OF KEROSENE STOVES?

No delicate specific qualitative test for ethylene in a mixture of gases was found. An attempt was made to isolate ethylene from stove gas by bubbling it through bromin water. This was done for periods of 6 to 10 days, about seven hours a day, on three different occasions, the absorbing tube being surrounded by ice in one case. No detectable quantity of bromid was obtained. It is believed, however, that ethylene, if present in stove gas, occurs there only in traces. If so, it would be difficult to detect it in this way. Thus, on the basis that 15 gm. of ethylene dibromid would be needed for the identification tests, if ethylene were present at a concentration of 1 to 1,000, it would be necessary to bubble stove gas through bromin for 24 hours a day for about 18½ days. At a concentration of 1 to 10,000 about six months would be required.

Aside from the fact that ethylene and stove gas produce similar effects upon green fruit, there are other reasons for believing that ethylene is present in stove gas. Ethylene has been found among gases that are produced in the cracking of petroleum (*11*). Denig (*4*) obtained ethylene as one of the products of the decomposition of kerosene by heat. According to Eldred and Mersereau (*7*), ethylene is produced by heating kerosene vapor under certain conditions. Lewes (*10*) states that ethylene is "found as one of the products in nearly all cases where organic compounds are subjected to distillation at high temperatures." A further fact pointing to the same conclusion was obtained when, in attempting to remove oxygen from stove gas by phosphorus, the phosphorus would not burn. White (*19*) states that "a fraction of a tenth of a per cent of ethylene will completely prevent the reaction between phosphorus and oxygen."

ETHYLENE FROM THE PRACTICAL STANDPOINT

EXPLOSIVE AND ANESTHETIC PROPERTIES

The sources, chemical behavior, physical constants, and important commercial uses of ethylene are discussed fully by Curme (*3*) and by Malisoff and Egloff (*13*). In connection with the experiments here reported two properties of the gas need to be discussed:

(*a*) When ethylene is mixed with air in proportions above 3 per cent by volume and below 30 per cent by volume, the mixture may be exploded by ignition. In liberating ethylene from the cylinder into a room containing fruit to be colored, however, explosive concentrations are not reached. To reach such proportions in a room of carload capacity, say 3,500 cubic feet, it would be necessary to liberate about 100 cubic feet of ethylene, or about one-third of the entire contents of a full cylinder. The lowest explosive concentration is at least 100 times as strong as the strongest application of gas needed for successful coloration.

(*b*) Luckhardt and Carter (*12*) have found that animals, including human beings, can be anesthetized by ethylene at a concentration of 80 per cent ethylene and 20 per cent oxygen. The effectiveness, however, decreases rapidly with decreasing concentration. Thus Smith and Hoskins (*18*) were unable to anesthetize or injure a mouse with 72.5 per cent ethylene and 27.5 per cent oxygen by exposure for one hour. The writer has freely breathed the pure gas as it comes from the cylinder and

has remained in the room for at least an hour after liberating a charge of gas and at no time has he noted any physiological effects. The concentrations necessary to produce anesthesia are about 800 to 8,000 times as strong as those recommended for coloring lemons.

COST OF ETHYLENE TREATMENTS

One cylinder of ethylene holds about 320 cubic feet of the gas at ordinary pressure and temperature. At present prices, the gas costs about 3⅓ cents per cubic foot, exclusive of the transportation charges and the value of the cylinder, which can be returned to the manufacturer for refilling. The cost of gas for coloring a carload of fruit should not exceed a dollar.

EFFECT OF MISCELLANEOUS GASES AND VAPORS UPON THE COLORATION OF GREEN LEMONS

During the course of the experiments, the effect of many substances upon green lemons was tried. A surprisingly large number caused yellowing of the fruit, but in most cases no practical application of the fact could be found, either because of injury to the fruit, expense of the reagent, its poisonous character, etc., or because the color developed was not the bright lemon yellow desired in commercial practice.

Among the substances that induced coloring but caused injury were paracresol, pyridin, ethyl butyrate, methyl amin, bromin, formaldehyde, acetaldehyde, chlorin, amyl nitrite, formic acid, nitric acid, and benzine. Substances causing coloration in strong concentrations but not in low concentrations were acetic acid, hydrochloric acid, trichlorethylene, and amyl acetate. Substances producing fairly good color without injury were gasoline, phenol, and chloral hydrate. Substances producing slight effects or none were calcium hypochlorite, hydroxylamin-hydrochlorid acid, alphanaphthylamin, guaiacol, sulphanilic acid, salicylic acid, benzoic acid, trimethylethylene, kerosene, turpentine, asphaltum, and ethyl ether. Methyl chlorid from a commercial cylinder at a concentration of 1 to 1,000 caused good coloration of lemons in 10 days, and that at a concentration of 1 to 100,000 caused slight coloring. In this case, however, it can not be stated that the methyl chlorid did not contain traces of ethylene.

SUMMARY

Some commercially mature lemons are green in color when picked. The desired yellow color must be secured by subsequent treatment.

When forced coloring is desirable, the change from green to yellow is hastened by the use of the combustion gases that arise from kerosene stoves during the burning of kerosene. The object of the investigation here reported was to determine the identity of the gaseous constituent responsible for the coloration of the fruit.

After the mixture of gases from a kerosene stove had bubbled through Pettenkofer tubes filled with bromin water, the residual (unabsorbed) gas failed to induce coloration. This suggested that the effective constituent must be among the unsaturated hydrocarbons.

Ethylene, even in low concentration, caused green lemons to turn yellow. Fruit colored in this way did not differ in any detectable manner

from similar fruit colored in the usual way with kerosene stoves. However, attempts to isolate ethylene from stove gas were unsuccessful.

When mixed with air in varying proportions by volume, concentrations of ethylene down to 1 to 200,000 colored lemons in 5 to 8 days. Concentration down to 1 to 2,000,000 required 6 to 10 days. The lowest concentration tried (1 to 5,000,000) required about 14 days, indicating that this concentration represents the highest dilution at which the rate of coloring is markedly influenced. High concentrations appear to retard coloring, since 80 per cent ethylene for 7 days gave only slight coloring.

Absence of oxygen prevented coloration of the fruit by ethylene.

Coloration was not measurably hastened by ethylene at a temperature of 45° F., but the rate of coloring increased with increasing temperatures from 57° to 82°. A reduction in rate, however, was observed at 93°.

Both ethylene and stove gas increased the rate of respiration of lemons. The carbon dioxid output was increased about 150 to 250 per cent.

Ethylene is now a commcercial article and can be bought as a compressed gas in steel cylinders. Gas in measured quantities may be released from the cylinder in a convenient manner and brought in contact with the fruit by use of an apparatus devised for the purpose.

The effect upon green lemons of many other substances, including carbon monoxid, acetylene, methyl chlorid, formaldehyde, acetaldehyde, pyridin, amyl acetate, and chlorin, was tested.

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NOCTURNAL PRODUCTION OF CONIDIA BY SCLEROSPORA GRAMINICOLA[1]

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INTRODUCTION

While studying the two conidial Sclerosporas (*S. philippinensis* Weston and *S. spontanea* Weston) so destructive to maize in the Philippines, the writer found that in these species conidia are produced only at night when the host surface is covered with dew or other moisture. This naturally raised the question whether *S. graminicola* (Sacc.) Schroet., the type species of the genus, exhibits the same peculiarity. The fact that it does and the bearing of this fact on our knowledge of the importance and relationship of the conidial stage of the species are discussed in the present paper.

The genus Sclerospora was established by Schroeter (*22*)[2] in 1879 on *S. graminicola*, a species which since then has been found quite widely distributed throughout temperate and tropical parts of the world, principally on species of Setaria, and rarely on other Gramineae. In the course of its life history *S. graminicola*, like most other Peronosporales, passes through two phases of development: The one, characterized by production of immediately germinating conidia, achieving rapid spread; the other, characterized by formation of resistant oospores, serving to insure survival through such unfavorable conditions as winter and drought. In *S. graminicola* the conidial stage, which usually develops first, appearing as a whitish downy growth on the surface of chlorotic areas of the host, generally seems to be of short duration, relatively inconspicuous, rather rare, and involves but little apparent injury to the host. As a result, this stage has not been commonly or abundantly collected and is represented by scanty and unsatisfactory herbarium material. Moreover, it has not been studied in detail. In species of such related genera as Plasmopara (*10*, *28*) and Peronospora (*6*, *7*, *8*), the conidial condition has been investigated intensively, quantitative measurements have been made, restrictions of parasitism have been tested, and morphological aspects of all stages of development have been worked out minutely and illustrated fully. In *S. graminicola*, however, the conidial stage, save in such publications as those of Butler (*2*), Kulkarni (*12*), Shirai (*24*), et al., has been dismissed summarily with brief diagnostic or morphological descriptions, few measurements, and inadequate illustrations.

On the contrary, the oosporic phase of *Sclerospora graminicola* which follows the conidial with a marked distortion and shredding of the leaves and floral parts of the host, is persistent, conspicuous, abundant, and obviously severely destructive to the host. As a result, it has been collected frequently and in abundance, and is well represented in most

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[2] Reference is made by number (italic) to "Literature cited," 783–784.

herbaria; while detailed study has been made on the morphology (*2*, *12*) and cytology (*26*) of its development, on comparative spore measurements (*27*), and on the relation to the host attacked (*2*, *22*, *23*).

Since the genus was founded, nine other species have been described. Of these, *Sclerospora macrospora* Sacc. (*18*), which includes *S. kriegeriana* Magn., according to Traverso (*27*), *S. miscanthi* T. Miyake (*16*), *S. farlowii* Griffiths (*9*), and *S. magnusiana* Sorokin (*25*), have been reported as yet only in the oosporic condition. The remaining five species are alike in that: First, their conidial phase is predominant and destructive, the oosporic being absent or rare; second, their conidia germinate invariably by hyphae; third, they occur in the oriental Tropics and on members of the tribes Maydeae and Andropogoneae. They are thus in contrast to the type species, *S. graminicola*, which is destructive and predominant in its oosporic condition, which typically produces zoospores in conidium germination, and which is of world-wide distribution, mostly on members of the Paniceae.

While investigating two of the conidial Sclerosporas of the Orient, namely, *S. philippinensis* and *S. spontanea*, species exceedingly destructive to maize in the Philippine Islands, the writer (*29*, *30*) found that in both, conidia were produced on the leaves of the infected plants only at night when they were covered with a layer of dew. On dewy nights, from about midnight to dawn, the innumerable successively emerging conidiophores formed a conspicuous and luxuriant growth of grayish down, and furnished abundant living material in all stages of development, quite different from the scanty remains, killed and deformed by drying, that persisted for collection or study during the following day. This nocturnal conidiophore production proved to be a very fixed and characteristic process in *S. philippinensis* Weston and *S. spontanea* Weston. Examination of the publications of other investigators suggested strongly that this condition holds also in the other conidial Sclerosporas of maize and related crops in the Orient, namely, *S. javanica* Palm (*17*) of Java, *S. maydis* (Rac.) Butl. (*3*) of India, and *S. sacchari* T. Miyake (*16*) of Formosa. Indeed, in the case of the last species, which has been introduced recently into the Philippines (*13*, *31*), there is now no doubt that conidium production is nocturnal, as material collected at intervals during the night and sent to the writer through the cooperation of H. Atherton Lee, of the Bureau of Science in Manila, shows this to be true.

PRODUCTION OF CONIDIA IN SCLEROSPORA GRAMINICOLA

Whether the type species, *Sclerospora graminicola*, also produces its conidia only at night is a question that naturally presents itself, in view of the fact that this species differs in the several respects already noted from the conidial Sclerosporas of the Orient which show this peculiarity. Opportunity to investigate this point occurred while the writer was attending the summer conference of cereal pathologists at the University of Minnesota in 1920. On the grounds of the College of Agriculture, not too far from the plant pathology laboratory which Dean Freeman and Doctor Stakman very generously made available for night work, were found several plants of *Setaria viridis* (L.) Beauv. obviously infected with the conidial stage of *Sclerospora graminicola*.

The fact that the tissue of these plants was pervaded extensively by vigorous mycelium of the parasite, and the leaf surface was marked by

whitish patches of shriveled conidiophores persisting on the leaves from previous productive nights, indicated that the plants were still supporting conidiophore formation under favorable conditions. Frequent careful inspection of these plants throughout the day of July 23, however, showed conclusively that, during this daytime at least, no conidiophore production took place. Accordingly, in the late afternoon, the plants were prepared for night study by removing from the leaves all remnants of previous conidiophore production by carefully scrubbing the surface with moist cotton swabs. That night, beginning at about 8 p. m., when dew deposition commenced, the plants were examined at hourly intervals and were found, indeed, to produce conidiophores in abundance.

By cutting free-hand sections of living, infected leaves, stripping off bits of epidermis, macerating pieces of tissue, and carefully scraping off the down of conidiophores as it developed, the process of conidiophore emergence and conidium production through the night was followed step by step in detail. After production had ceased, as the plants dried off in the early morning of the next day, periodic examination of the plants was continued until afternoon; and this again brought out the fact that no conidiophores were produced during the day. The fact that in *Sclerospora graminicola* conidium formation does indeed occur at night was established by these observations; but in order to supplement them, as they extended over two days and one night only, the most vigorous of the infected Setaria plants were transplanted to Washington, D. C., where they were studied further.

The process of conidiophore production involves, as the writer found in the conidial Philippine Sclerosporas, the following phases: The preliminary paling of the leaf areas which are to bear conidia, the quantity of conidia produced on such areas, the length of time this production may go on night after night, the importance of moisture in inducing conidiophore production, the development of conidiophores and conidia thus induced, and the nightly schedule followed by this development. The writer did not have opportunity to work out in detail these several points for *S. graminicola* as he did in the Philippine Sclerosporas, but studied chiefly the development of the conidiophores and conidia, the nightly schedule followed by this development, and its dependence on dew or similar moisture. The results of this study follow.

DEVELOPMENT OF THE CONIDIOPHORES

The conidiophores of *Sclerospora graminicola* develop from the infected Setaria leaves only through the stomata, a large proportion of which may be productive. Consequently, conidiophore production is usually more abundant from the under surface of the leaf where stomata are more numerous, although this abundance is dependent also on other factors such as the amount and distribution of dew on the leaf and the length of time successive nocturnal production from the plant has gone on. Beneath stomata from which conidiophores are to emerge, the air chamber is filled with stout, irregularly lobed, densely granular, mycelial branches closely crowded together (Pl. 1, A, B), arising from the less conspicuous mycelial strands running between the mesophyll cells. From these substomatal knots, prolongations push through the stomatal slit over which they form a compact group of several minute bulbous outgrowths. In some cases there is evidence that the stomatal slit, normally closed at night, is forcibly pushed open by these emerging branches

(Pl. 1, C–F). The bulbous outgrowths next elongate severally to club-shaped stalks which project perpendicularly from the leaf surface and grow rapidly larger (Pl. 1, G, H). At its swollen apex, each of these produces successively the two to four stout primary branches (Pl. 1, I–K); while from these latter similarly arise the secondary branches (Pl. 1, L–O). In like manner, successive series of branches develop until eventually the more or less extensive, usually dichotomous, branch system is complete, and the ultimate tips terminate in tapering sterigmata (Pl. 2, A). The very apex of each sterigma, beginning as a small globular swelling (Pl. 2, B–E), enlarges gradually, until finally it attains the shape and size of the mature conidium (Pl. 2, F), which then is separated from the neck of the sterigma by a wall.

Obviously, the development of the conidiophores and conidia in *Sclerospora graminicola* as just outlined agrees very closely in its several stages with that already established for *S. philippinensis* and *S. spontanea*. Moreover, *S. graminicola* apparently agrees with these Philippine species in the way in which its conidia are shed. While studying the Philippine species, the writer became convinced that the conidia do not fall passively from the sterigmata as has been assumed, but rather are forcibly snapped off when the outbulging of the opposed walls of the basal apiculus of the conidium and of the sterigma tip suddenly overcomes the adhesion of their contiguous surfaces. This point has not been settled for *S. graminicola;* but such indications as the outbulging of the formerly flatly apposed walls of the sterigma tip and conidium base when released, lead to the conclusion that the species resembles those of the Philippines in this respect also.

DEPENDENCE ON NOCTURNAL MOISTURE

The process of conidiophore development in *Sclerospora graminicola* adheres to a regular schedule. When the infected leaves were wet with dew, at about 8 p. m., the outgrowths had protruded from the stomatal slit at about 11 p. m., and the first conidiophores and conidia were mature at approximately 2 a. m., while others which had begun to develop meanwhile, matured successively, so that production continued, reaching its greatest abundance at perhaps 3 a. m., and only ceasing when the dew dried from the leaves in the morning sun. This nightly schedule agrees very closely with that found in *S. philippinensis* and *S. spontanea* (*32*) under conditions of dew deposition normally obtaining in the Philippines.

Conidiophore production in *S. graminicola*, as in the Philippine species, is vitally dependent on the presence and persistence of dew or other moisture on the leaves. Infected Setaria plants that were kept dry during the night never showed conidiophore formation even though their leaves were obviously thoroughly invaded by vigorous mycelium, while similar plants exposed to dew supported abundant conidiophore production. After the leaf surface of infected plants had been wet with dew for about five hours, production began; if the moisture dried off prematurely, production coincidentally ceased; if moisture persisted unduly into the morning, production was thus much prolonged. As in the Philippine Sclerosporas also, conidium production in *S. graminicola* is very sensitive in its response to moisture changes, so much so that,when studied at the University of Minnesota, the Setaria plants in such different localities as in a glade among trees, at the edge of a wood on a small hill, and in an open lot, showed slight variations in their schedule of

conidiophore production as a result of local differences in amount or time of dew deposition.

How long *Sclerospora graminicola* may continue nocturnally producing conidia on infected Setaria plants, how tolerant of this continued production the host may be, and how much of its life span may be taken up by the conidium-forming period of the fungus, are points which, in this case, were not worked out in detail as they had been for the Philippine Sclerosporas (*32*). In one case, however, even though the transplanted Setarias grew very poorly, one plant showed intermittent conidiophore formation for more than two weeks. Also, in the field, there is abundant evidence that the period of conidium production continues much longer than this, even extending over as great a proportion of the total life of the host as did the Sclerosporas studied by the writer (*32*) on maize in the Philippines.

A study of large numbers of *S.-graminicola*-infected Setaria plants of various ages in the fields near the College of Agriculture at St. Paul, Minn., showed the progress of the disease to be as follows: Production of conidia begins on newly unfolding leaves in symptomatically chlorotic streaks which, by their extent and position even in very young plants, indicate a fundamental systemic infection starting in the young seedling and giving rise to thorough and extensive invasion of the host tissue. The production of conidia continues during each favorable night on these earlier leaves, and also continually starts up afresh on leaves successively appearing. Gradually, as the host matures, conidiophore formation from the host surface is superseded by the development of oogonia on the intramatrical mycelium, beginning in the lower, first unfolded, older leaves, and working slowly upward. The uppermost, latest, and youngest leaves are the last to be affected, and may continue abundant conidiophore production until the head, usually deformed, sterile, and virescent, is full grown. Finally, however, even these ultimate leaves also are given over to oogonium formation, and show the shredding of tissue which marks the maturity of the oogonial phase of the fungus.

The conidial stage of *Sclerospora graminicola*, because its relation to nocturnal moisture has not been understood, has been considered from the time of Schroeter to the present as fleeting, transitory, fugacious, and evanescent. Now, however, that we know the period of conidium production may be of relatively long duration, we must alter our conception of the evanescence of the conidial stage. It is fugacious in the sense that conidiophore production takes place for only a few hours during the night; but it is decidedly persistent in the sense that this production may go on night after night throughout a large proportion of the total life of the host.

CONIDIAL SCLEROSPORA GRAMINICOLA COMPARED TO OTHER SPECIES

Material of the conidiophores and conidia of *Sclerospora graminicola* gathered thus at night during the period of optimum conidiophore production is infinitely more satisfactory for study than the dried and shriveled remnants which persist from previous nights and may be collected during the day. Consequently, such material gives a somewhat broader conception of the character of the species than that which we gain from the usual descriptions and figures based on dried material, and also permits a more adequate comparison with the conidiophores and conidia of other species.

CONIDIOPHORES

In the case of the conidiophores, for example, a study of optimum nocturnal material shows clearly that the conidiophores are larger, more extensively branched, and structurally more complex and elaborate, than most of the illustrations and descriptions indicate. The first description of the conidial stage of this species was published by Schroeter (*22*) and the first illustrations were shown by Fischer (*5, fig. 71*). Together these present a vivid characterization of the conidiophores as decidedly short (about 100μ) and thick (about 12μ) with few stubby branches bearing a relatively small number of conidia (about 15). No appreciable departure from this characterization is found in the publications by such subsequent investigators in Europe as Berlese (*1*), Malbranche and Letendre (*14*), Massee (*15, pl. 1, fig. 16*), Saccardo (*19*), et al.; or such in America as Saunders (*21, pl. 16, fig. 4*), Farlow (*4*), Wilson (*33*), et al.; or in India as Butler (*2, fig. 6, 7*), and Kulkarni (*12, fig. 1–3*). In Japan, however, Shirai (*24, fig. 16, 17*) described the conidiophores as much larger (100 to 240μ by 12 to 19μ) and with a somewhat more elaborate branching system; but the fact that he found conidia of two strangely different size-classes (24 to 28.8μ by 16.8 to 19.2μ, and 38.4 to 57.6μ by 19.2 to 24μ) arouses the suspicion that he was not dealing with *S. graminicola* alone, and to some extent invalidates his characterization of the species.

The writer, after studying the conidiophores of *Sclerospora graminicola* in the progressive stages of their development under optimum conditions at night, is convinced that the descriptions and drawings of the investigators just mentioned are based on nontypical, poorly developed specimens, the last belated stragglers of the nocturnal production, caught by the morning sun before they had developed conidia, and dried to a condition still less typical and favorable for study. In contrast to such dried specimens, material scraped from Setaria leaves at the time of optimum nocturnal production shows conidiophores that are much larger and better developed than we had been led to believe characteristic of *S. graminicola*, and which approximate, in this respect, those of such luxuriant species as the conidial Sclerosporas of the Orient. If, for example, one compares the accompanying figures of *S. graminicola* (Pl. 2, I, K, O) with those of *S. spontanea* (*30, pl. 79, fig. A*), and *S. philippinensis* (*29, pl. 24, fig. O, pl. 25, fig. B*) all of which are of very nearly the same magnification, it is obvious that they show a general resemblance which is not even suggested by previous illustrations.

After comparing them carefully, however, the writer finds that even the largest and most elaborate conidiophores of *S. graminicola* differ markedly in certain essential features from the conidiophores of such oriental species as *S. philippinensis* and *S. spontanea*.

First, the total length in these Philippine species is as a rule much greater (260 to 400μ) than in the case of *S. graminicola*, even the most luxuriant nocturnal material of which has a length of about 150μ with occasional extremes as low as 100μ or as high as 200μ.

Second, the branching system also shows differences which are much more qualitative and absolute than are mere distinctions in size. In the Philippine species, for example, usually three, sometimes two or four, primary branches of approximately equal size and extent of development all spread out at angles of about 45° or less from the main axis in very close succession; and all are of equal rank—no one of them being either in direction, position, or extent of growth more to be considered a continua-

tion of the main axis than any other. As a result of this also, the conidia arising from the branch tips are arranged approximately in a hollow hemisphere. In *S. graminicola*, on the contrary, one of the primary branches stands out more or less obviously as a continuation of the main axis (Pl. 2, I, K, L, O, Q) both in direction and in extent of growth. From this continuation of the main axis other main and secondary branches grow out at irregular intervals, usually at angles of 45° to 90°. As a result, the conidia at the ends of the branches lie more frequently in irregularly disposed groups (Pl. 2, I, K, L, P) than in an approximate hemisphere (Pl. 2, O, Q).

Third, the sterigmata of *S. graminicola*, as a rule, are shorter and more broadly bottle or tenpin shaped (Pl. 2, N, T, V, W) than are those of the Philippine Sclerosporas. The shape, however, is somewhat dependent on position, single sterigmata at the tips of the branches (Pl. 2, U) being more elongate than those borne in groups of two, three, or four (Pl. 2, T, J, W). Moreover, the sterigmata, which in the Philippine Sclerosporas almost invariably continue the direction of the branch tip that bears them, may stand out from the branch tip even at right angles in *S. graminicola* (Pl. 2, I, J, L). Finally, the lower part of the conidiophore of *S. graminicola* is not usually marked off by a cross wall into a basal cell or foot cell that is distinct from the superior portion of the main axis. Occasionally such basal cells are encountered (Pl. 2, K, X, Y); but this condition is the exception; and hence is in distinct contrast to the Philippine species in which it is a characteristic feature. Occasionally, also, the base is distinguished by an incomplete transverse septum (Pl. 2, K) or by a decided thickening of its longitudinal wall (Pl. 2, A, P, Z).

All these distinctions—size of the conidiophores, character and extent of the branch system, form and direction of the sterigmata, and extent of septation or thickening at the base of the main axis—are matters of degree which should be expressed quantitatively to facilitate comparisons. Yet, even when considered as qualitative differences, they show clearly that the conidiophores of *S. graminicola*, though they may approximate those of such typically conidial oriental forms as *S. philippinensis* and *S. spontanea* in luxuriance and general appearance, are indeed distinct from them.

CONIDIA

It is the conidia themselves, however, that are the most distinguishing feature of *Sclerospora graminicola*. These bodies differ markedly from those of other Sclerosporas in size, shape, structure, and germination. The size of the conidia varies, and for adequate presentation requires quantitative expression based on measurements of large numbers of conidia. Moreover, to be ideally satisfactory the conidia should be caught on the slide in dew when snapped off from the conidiophores at maturity and measured at once. Unfortunately, the writer was not able in this case to make all the measurements under these ideal conditions as he did for the Philippine Sclerosporas. Rather, most of the measurements were made from material scraped from abundantly productive leaves at 3 a. m., then killed by Flemming's weaker solution, and mounted in dilute glycerin and eosin. A comparison of measurements made thus with the relatively few which the writer had opportunity to make from fresh material indicates that if all the measurements had been made under ideal conditions the modes of length and of diameter probably would have been

increased by one 2μ class (from 18 to 20μ and from 14 to 16μ). With this probable correction in mind, however, the 400 measurements [3] here pre-

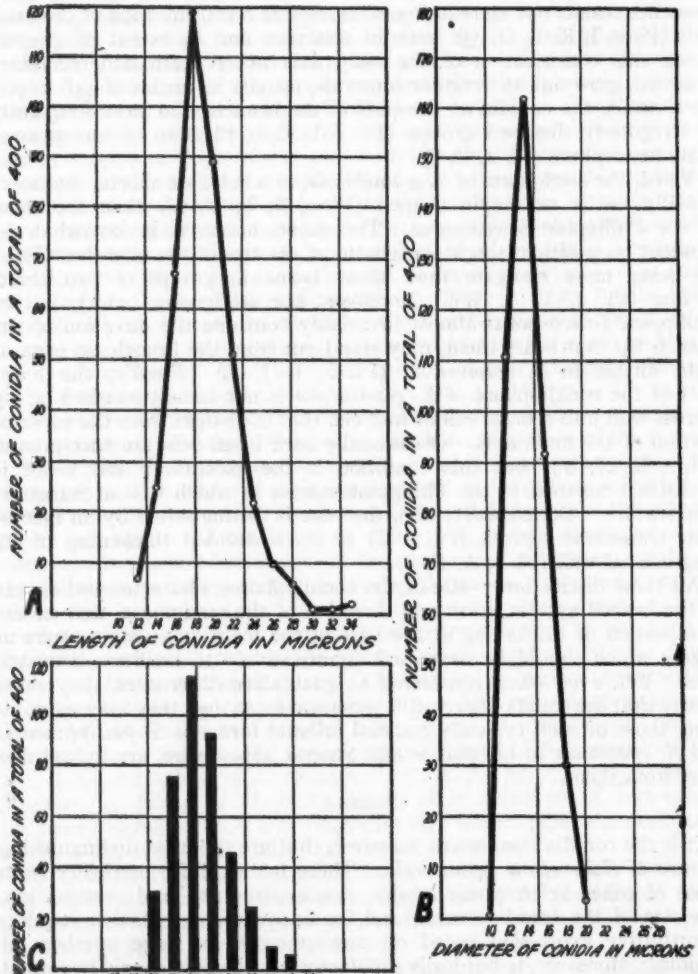
NUMBER OF CONIDIA IN A TOTAL OF 400
A
LENGTH OF CONIDIA IN MICRONS
NUMBER OF CONIDIA IN A TOTAL OF 400
B
DIAMETER OF CONIDIA IN MICRONS
NUMBER OF CONIDIA IN A TOTAL OF 400
C
CLASS
RATIO OF LENGTH OVER DIAMETER
U.S. DA

FIG. 1.—Diagrams showing the frequency of occurrence of various sizes encountered in 400 conidia (zoosporangia) of *Sclerospora graminicola;* A, variation of the conidia in length; B, variation of the conidia in diameter; C, ratios of length to diameter.

sented (fig. 1 and Table I) offer a fair basis of comparison with other species.

[3] The writer is indebted to Miss Margaret Kemp, graduate student in botany at Radcliffe College, for making one half of the measurements embodied in the table and graphs.

TABLE I.—*Measurements, and ratios of length to diameter of 400 conidia (zoosporangia) of Sclerospora graminicola arranged in size and ratio classes*

Length.		Diameter.		Ratio, length to diameter.	
Classes.	Number of conidia in 400.	Classes.	Number of conidia in 400.	Ratio classes.	Number of conidia in 400.
μ		μ			
11 to 12.9	7	9 to 10.9	1	0.95 to 1.04	1
13 to 14.9	25	11 to 12.9	111	1.05 to 1.14	31
15 to 16.9	67	13 to 14.9	162	1.15 to 1.24	75
17 to 18.9	119	15 to 16.9	92	1.25 to 1.34	115
19 to 20.9	89	17 to 18.9	30	1.35 to 1.44	92
21 to 22.9	51	19 to 20.9	4	1.45 to 1.54	46
23 to 24.9	22			1.55 to 1.64	25
25 to 26.9	10			1.65 to 1.74	9
27 to 28.9	6			1.75 to 1.84	6
29 to 30.9	1				
31 to 32.9	1				
33 to 34.9	2				

From the measurements presented above in graphic and tabular form, it is obvious that, although conidia are encountered which measure from 12 to 34μ in length and 10 to 20μ in diameter, the greater proportion of them are between 16 and 22μ long by 12 to 16μ in diameter. These measurements present an interesting comparison to those previously recorded for *S. graminicola*. In Europe, Schroeter (*23, p. 115*) gave the size of the conidia as 20μ long by 15 to 18μ wide, while Saccardo (*19*), apparently using scanty and immature material, first recorded 12 to 15μ by 10 to 11μ, and later (*20, p. 238*) modified this to 12 to 20μ by 10 to 18 μ to include Schroeter's figures. Others, such as Malbranche and Letendre (*14*), Fischer (*5, p. 437*), and Berlese (*1, v. 9, p. 70*) followed these two without making original measurements themselves. In the United States, Saunders (*21, p. 58*) is apparently the only one to give original measurements, 18 to 20μ by 13 to 15μ, the others, from Farlow (*4*) to Wilson (*33*), following Schroeter and Saccardo. In the Orient, Butler (*2*) gave 22 to 30μ by 12 to 16μ and Kulkarni (*12*) 19 to 31μ by 16 to 21μ for *S. graminicola* in India; while Shirai (*24*) for Japan, gave 24 to 28.8μ by 16.8 to 19.2μ for the usual size, but made the startling statement that at times giant conidia, 38.5 to 57.6μ by 19.2 to 24μ, occurred. Although no exact comparison can be made with inclusive, limiting, nonquantitative measurements such as these, there seems to be a fair agreement between the European and United States figures and those of the writer. The measurements from the Orient apparently show a consistently greater length; but whether this indicates different specialized races or merely greater luxuriance of growth under tropical conditions remains to be determined. The remarkable discrepancy in size and shape reported by Shirai arouses the suspicion that he was dealing with two species, and demands thorough corroboration before this larger size is included as a correct measurement for *S. graminicola*.

In shape, the conidia vary considerably, ranging from subspherical through ovoid and obovoid to ellipsoid, lemon-shaped, and rounded-

cylindric. Fully mature spores are perhaps most commonly broadly ellipsoid or broadly cylindric. A qualitative idea of the usual variations in form may be gained from the representative conidia grouped in Plate 2, M. A quantitative idea of the relative predominance of the short, broad shape is given by the ratios of length to diameter which are grouped in Table I (p. 779) and in the diagram (fig. 1).

Obviously, both in their small size and rotund shape the conidia, as revealed in this nocturnally collected material, mark *Sclerospora graminicola* as distinct from the other conidial Sclerosporas—namely, the typically ellipsoid, large-spored species of the Orient. *Sclerospora javanica* of Java, however, if we may judge from the 20 measurements given by Palm (*17*), has rotund conidia ranging from 22 to 26μ in length by 16 to 20μ in diameter, but most commonly 24 by 18μ, a size so closely approaching that of the conidia of *S. graminicola* reported from Japan by Shirai (*24*) and from India by Butler (*2*) and Kulkarni (*12*) that distinction based on conidia alone might be difficult were it not for the considerably different structure and germination of these spores.

The wall of the conidium is thin (0.5–1μ) and of cellulose save at the apex where, as the conidium matures, the single so-called papilla of dehiscence develops (Pl. 2, G, M, O, T). This is a specialized tip, approximately plano-convex in shape, although of variable thickness and area, and of modified cellulose (pectin or hemicellulose) composition. At the opposite (basal) end of the conidium there persists in some cases an apiculus of attachment (Pl. 2, G), which is merely the point at which the conidium was affixed to the sterigma and is not at all comparable to the apical papilla.

At germination the apical papilla of dehiscence softens and gelatinizes, leaving a terminal pore (Pl. 2, H) through which escape the several zoospores into which the granular conidium content has by then become differentiated. The conidium is thus in effect a zoosporangium, but like many other Phycomycete zoosporangia it has the potentialities of a conidium and may, under circumstances unfavorable to zoospore emergence, germinate by sending out hyphae. Under ordinary circumstances, however, hyphal germination is rare, the writer having seen only a few instances among many observations. Also, even when germinating by hyphae, the conidia are distinguished by the presence of the apical papilla of dehiscence from conidia of other species of Sclerospora in which hyphal germination is the rule.

The process of germination, involving as it does the maturing of the zoosporangium, the development of the zoospores, the formation of the apical papilla of dehiscence, the deliquescence of this structure, and the escape and subsequent behavior of the zoospores, shows many points of interest which the writer hopes to work out in detail and present at a later date.

It is the conidia, then, as they appear in favorable night-collected material which particularly distinguish the conidial stage of *Sclerospora graminicola*. Their short, thick form and small size, although relative characters, are of some diagnostic value in distinguishing the species. The possession of an apical papilla of dehiscence, however, is the one absolute distinction. By this character, and secondarily by the zoosporic germination which usually follows, *S. graminicola*, as yet, must stand as a unique representative of the genus. In this connection, Kulkarni's (*12*) study on this species in India is of especial interest. This investigator found that, although the characteristic oogonial stage of *S. graminicola*

on *Pennisetum typhoideum* Rich. and *Andropogon sorghum* (L.) Brot. is apparently the same on both hosts, the conidial stage found on the latter host differed markedly from the typical conidial *S. graminicola* which developed on Pennisetum. Conidia produced on *A. sorghum*, by their distinctly subspherical shape, absolute lack of an apical papilla of dehiscence, and invariable germination by tubes, consistently present, even though agreeing in size, a very decided contrast to the broadly elliptic, apically papillate, zoospore-forming conidia of the type. Moreover, the branch system of the Andropogon fungus was more extensive than that of the type, and the sterigmata reached a length of 16.3μ while those of the type were but 8.3μ. Also, the fungi showed differences in their effect on the two hosts in the field and in their failure to produce cross infection. As a result, Kulkarni establishes the fungus on *A. sorghum* as *S. graminicola* var. *Andropogonis sorghi*.

In the opinion of the writer, this is certainly a distinct species, one which, aside from other differences, needs only the absolute criterion that its conidia lack an apical papilla of dehiscence to distinguish it without question from *S. graminicola*. It is clearly a species closely allied to the destructive, predominantly conidial Sclerosporas of the Orient even though it is apparently connected with an oogonial stage, presumably that of *S. graminicola*. Also, it promises most interesting results if studied intensively through numerous cross inoculations, comparative measurements of large numbers of conidia, and persistent efforts to determine whether it is or is not actually genetically connected with the typical *S. graminicola* oogonia on various hosts. Even before such an investigation is made, however, we are, in the writer's opinion, justified in regarding the apical papilla of dehiscence of the conidia of S. *graminicola* as a diagnostic feature of absolute value—a feature as yet confined to this species alone.

GENERAL DISCUSSION

Because it differs, in the respects which have been discussed, from all other known conidial members of the genus, there is the more significance and interest in the fact that *Sclerospora graminicola* shows the same main features of nocturnal conidiophore production which characterize at least three of these other conidial species. This fundamental agreement in behavior, together with the general similarity in structure and development, is sufficient, in the opinion of the writer, to outweigh the difference in germination. It seems undesirable, therefore, to establish a new genus on the species whose conidia germinate by hyphae, at least until much more extensive comparative study of the several species has given us further basis for such a rearrangement. Within the genus, however, there might be advantages in following Ito's (*11*) suggestion of establishing one subgenus, Eusclerospora, to include *S. graminicola*, and another, Peronosclerospora, to comprise the species the conidia of which germinate directly.

Of the Sclerosporas with known conidial stages, four species, *S. philippinensis*, *S. spontanea*, *S. sacchari*, and *S. graminicola*, alike have been found by the writer to be characterized by nocturnal conidiophore production. It will rest with future investigation to justify the natural assumption that this feature is common to all conidial Sclerosporas. It will rest with future investigation also to decide with finality what factor or combination of factors is operative at night to induce conidiophore

production. These observations on *S. graminicola* corroborate the writer's earlier decision (*32*), that persistent dew or other moisture on the infected leaves is of primary importance. More precise physiologic study probably will show, however, that the relationship is more subtle and complex than this would imply.

In any case, now that we know that *Sclerospora graminicola* produces its conidia only at night when the infected leaves are covered with a layer of moisture, we are in a position to study the dispersal of the conidia, their relation to the dissemination of the species, and the part the conidial stage as a whole plays in the life history of the fungus; to investigate intensively the physiologic and morphologic aspects of its parasitism, and the immunity or susceptibility shown by more or less related hosts; in short, to determine facts of immediate application toward the control of this destructive parasite.

SUMMARY

In the peronosporaceous genus Sclerospora, oogonial and conidial stages are known. Both of these develop regularly in the type species, *S. graminicola* (Sacc.) Schroet. Of the nine remaining species four are known as yet only in the oosporic condition. All the other five alike are predominant and destructive in their conidial phase—the oogonial being absent or very rare; all show germination of the conidia by hyphae; and all occur in the oriental Tropics on grasses of the tribes Maydeae and Andropogoneae.

To these five conidial species, *S. graminicola* is apparently in distinct contrast; for, although it does, indeed, develop a conidial stage, the oogonial condition is the predominant and obviously destructive one; the conidia (zoosporangia) germinate by zoospores; and the distribution is world wide—the host plant usually being a grass of the tribe Paniceae (Setaria, etc.).

Despite these differences, however, the writer finds that the conidial phase of *Sclerospora graminicola* shows certain of the same fundamental features of development which he has found recently to be always present in three of the five typically oriental species. The first fundamental feature is that *S. graminicola* produces its conidiophores only at night and when the surface of the infected leaves is covered with a layer of dew or similar moisture.

The second fundamental feature is that this production of conidiophores runs a well-defined course as follows: The knots of stout hyphae crowded in the substomatal air chambers push out prolongations through the stomatal slit, and form bulbous outgrowths which elongate successively to clavate stalks; and these in turn develop at their tips a more or less extensive branch system, and ultimately sterigmata and conidia.

The third fundamental feature is that this process of conidiophore production follows a regular nocturnal schedule which is vitally dependent on the presence and persistence of dew or other moisture on the leaf surface.

The writer describes and illustrates conidiophore development in *S. graminicola* and compares the species with *S. philippinensis* and *S. spontanea* in the regularity and dependence on moisture of its nocturnal schedule. The fact that conidiophore production is nocturnal only, that during the day there remain only remnants of the previous night's

crop, and that the spores and conidiophores can not survive desiccation explains why so little concerning the conidial stage has been known hitherto.

When studied in a fresh condition as they form at night, the conidiophores are found to be larger and much more elaborate and complex than one would have assumed from previous descriptions and illustrations—even approximating in luxuriance the conidiophores of the typically conidial species of the Orient. Nevertheless, *S. graminicola* stands as distinct from all other species now known. Its conidiophores have certain essentially characteristic features; while its conidia each develop an apical papilla of dehiscence, and hence germinate by emitting zoospores.

The fugacity of the conidial stage has been overemphasized heretofore; for, although it is fugacious in the sense that conidiophores develop only for a few hours each night, yet it is distinctly persistent in that this development may be repeated night after night for a considerable part of the life of the host.

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PLATE 1 [a]

A.—Mycelium of *Sclerospora graminicola* within a portion of a cross section of a badly infected *Setaria viridis* leaf cut at 10 p. m. The invading hyphae have pushed the mesophyll cells apart, and knobby branches have made their way into the air chamber under the stoma through which they will grow out and give rise to conidiophores. At *a*, a small, bulbous haustorium has penetrated an epidermal cell, and at *b* a branch of the mycelium appears in cross section as it runs between the mesophyll cells at right angles to the section. × 425.

B.—A bit of Setaria leaf like that shown in A, but more highly magnified, and cut in longitudinal section somewhat later at night. The mycelium has invaded the tissue more extensively, and has developed in the air chamber a crowded group of proliferating branches, some of which are just about to push out through the stomatal slit. × 850.

C.—Exterior view of a stoma in a piece of epidermis cut from an infected leaf of *Setaria viridis* at midnight. A knoblike protrusion has grown out from the proliferating branches, which are shown crowded in the substomatal air chamber in B, and is pushing through the partly closed stomatal slit ready to elongate into a conidiophore initial. 12 p. m. × 375.

D–E.—Early stages in the development of the conidiophores of *Sclerospora graminicola* from the leaves of *Setaria viridis*, showing the elongation of the knoblike outgrowths, seen in C, into club-shaped conidiophore initials. 12 p. m. × 375.

F.—Exterior view of a bit of the epidermis of a Setaria leaf, looking obliquely at a clump of young conidiophore initials arising from the almost obscured stoma, and showing various stages of development, from young knoblike outgrowths that have just emerged to older elongate stalks. At *a* is shown the base of a conidiophore already matured, and now shriveled and broken off; and, at *b*, a stoma as yet uninvaded by the fungus. 12 p. m. × 375.

G–O.—Later stages in the development of the conidiophores, showing the formation of the successive series of branches (primary, secondary, etc.), until finally at O the branch system is complete. 2 to 3 a. m. × 375.

P.—A bit of the upper epidermis cut from an infected Setaria leaf at 3 a. m., and showing, in oblique view, a maturing conidiophore. This conidiophore is an unusually stunted one, bearing only eight still spherical and not yet mature conidia, on a reduced branch system of two primary branches each of which gives rise directly to four sterigmata and conidia. 3 a. m. × 375.

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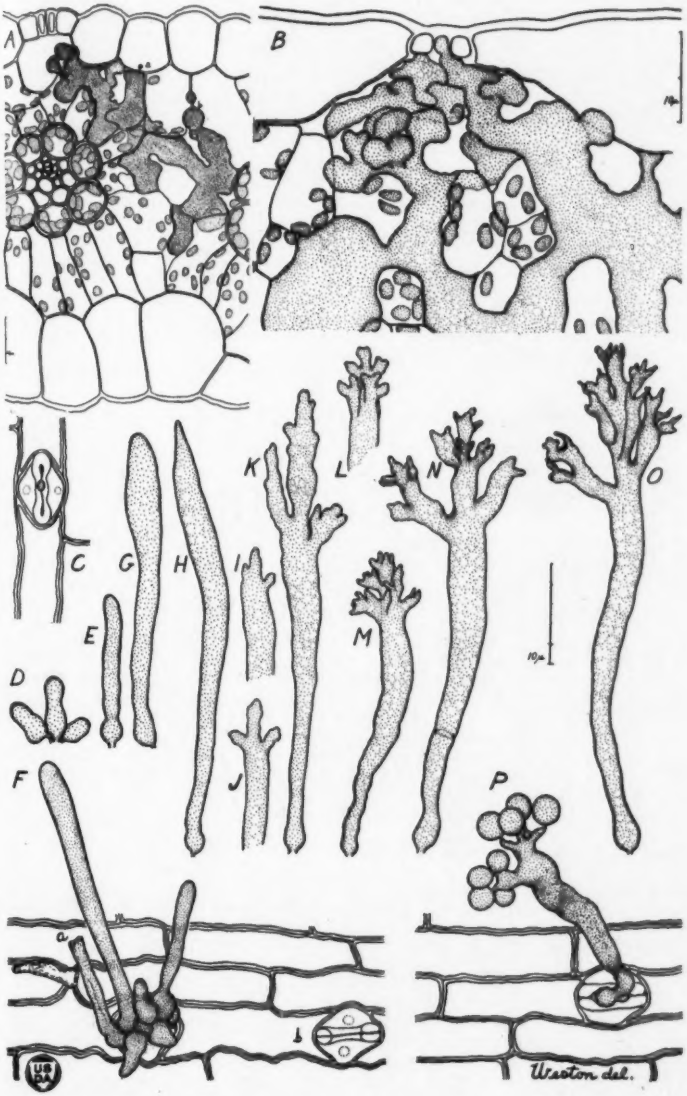
[a] The drawings were made with the aid of a camera lucida, and are all of *Sclerospora graminicola* on *Setaria viridis*. A, B, C, F, and P are drawn from free-hand sections of living material cut at night in water, and immediately killed and fixed in Flemming's weaker solution, stained, and mounted. D, E, and G to O are from material carefully scraped from productive leaves at night, and either drawn at once while still living, or drawn after being killed and mounted as were the sections. The approximate magnification of the printed figure after its reduction from the original drawing is given in each case, as is also a scale with 100 divisions as an absolute measure.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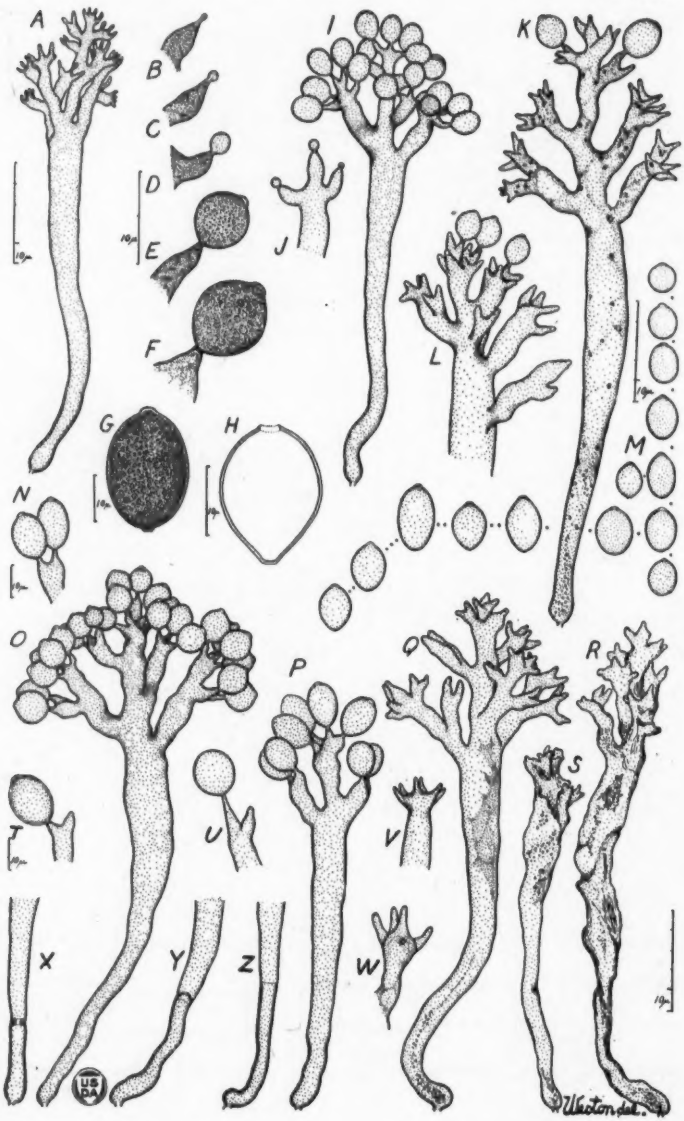
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PLATE 2 [a]

A.—A conidiophore of *Sclerospora graminicola* with the branch system completed and its ultimate tips elongating into sterigmata which will give rise to the conidia. The wall at the base of the main axis is thickened into a differentiated, footlike portion. × 375.

B–F.—Successive stages in the development of the conidia from the tips of the sterigmata. Note that at a relatively early stage, the wall at the tip begins to thicken and becomes modified into the characteristic apical papilla of dehiscence. × 850.

G.—Mature, recently shed, conidium with well-developed papilla of dehiscence at its apex, and the small apiculus by which it was attached to the sterigma still persistent at the base. The content has not yet begun to divide into zoospores, but is still undifferentiated and granular. × 850.

H.—The empty wall of a conidium (i. e., zoosporangium) which has germinated by emitting zoospores through the pore left by the softening and dissolution of the terminal papilla of dehiscence. × 1,200.

I.—Conidiophore at a later stage than that shown in A, with a vigorous, well-developed branch system which bears 18 only partly developed conidia. These conidia are still spherical, and have not as yet developed the terminal papillae of dehiscence. × 375.

J.—Detail of a branch tip of the three-pronged type frequently encountered (cf. fig. 1). Note that the young conidia which are just beginning to develop from the three sterigmata are not of exactly the same stage of development. × 600.

K.—A large, well-developed conidiophore which has shed all but two of its conidia. The branch system shows a somewhat extreme case of the continuing of the main axis, a characteristic of *Sclerospora graminicola*. An attempt has been made to show the detail of the content as it appears when the conidiophore is at this stage. An incomplete septation at the base delimits the basal cell which is occasionally encountered in this species. × 375.

L.—A still more extreme case of continuation of the main axis in the branch system of the conidiophore. × 375.

M.—Representative conidia showing the various shapes and sizes most frequently encountered. Note that each has a terminal papilla of dehiscence. The content, which is conventionally stippled here, is shown in detail in G. × 375.

N.—Detail view of two conidia still in place but already mature and about to be shed from the sterigmata. × 600.

O.—Typical, vigorous, well-developed conidiophore, like that in figure 1, but in a later stage of development. This is shown by the maturity of its 24 conidia, their larger size, more rotund ellipsoidal shape, and prominent papillae of dehiscence. Note the absence of any foot cell or basal thickening of the main axis wall. 2 a. m. × 375.

P.—A small, stunted conidiophore. The branch system, in comparison to that of a typical well-developed individual such as the one shown in O, is much reduced. Its three primary branches give rise directly to eight sterigmata and conidia, which, however, are larger than the more numerous ones of O. Note that the wall of the main axis is thickened at its base. 3 a. m. × 375.

Q.—A typical, well-developed conidiophore which only recently has shed its 34 conidia, as is shown by the bottle-shaped and rounded tips of its sterigmata, and by the beginning of the disintegration of its content. 3 a. m. × 375.

R.—The shriveled and shrunken remains of such a conidiophore, which had developed during some previous night, and had remained, dried, on the leaf until scraped off and examined during the day. × 375.

S.—A similar, mummied conidiophore, but one more completely shrunken and collapsed after longer drying. × 375.

T.—Detail of a branch tip of the dichotomous type so frequently encountered. One sterigma still bears a mature conidium; while the other by its bulging, rounded apex shows that it has only recently discharged.

U.—Detail of a similar branch tip with one typical, short sterigma which has already shed its conidium, and one rather unusually elongate sterigma bearing a conidium only partly developed. × 600.

V.—Detail of a branch tip with six sterigmata which have a somewhat uncommon arrangement resembling slightly that of the sterigmata of Bremia. × 600.

W.—A branch tip of a type frequently encountered, showing four approximately equal pronglike sterigmata standing out at equal distances from the club-shaped branch tip. × 600.

X–Y.—Typical examples of the kind of basal cells occasionally found in conidiophores of *Sclerospora graminicola*. × 375.

Z.—Base of a conidiophore showing the thickened wall that at times marks off a differentiated foot portion instead of a basal cell. × 375.

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[a] The drawings were made with the aid of a camera lucida, and are all of *Sclerospora graminicola* on *Setaria viridis*. Figures H and I were drawn from living material; the others from material scraped from leaves bearing abundant conidiophores at the time of maximum nocturnal production, and immediately killed with Flemming's weaker solution, stained (chiefly with eosin), and mounted in dilute glycerin. The approximate magnification of the printed figure after its reduction from the original drawing is given in each case, and also a 10μ scale is shown as an absolute measure.

DAILY VARIATION OF THE CARBOHYDRATES IN THE LEAVES OF CORN AND THE SORGHUMS [1]

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INTRODUCTION

In a comparative study of the physiological functions of corn (*Zea mays*) and the sorghums (*Andropogum sorghum*), it was thought advisable to make some observations on the variations of the carbohydrates in the leaves of these plants during a 24-hour period. It was considered that any data obtained in a study of this kind would be of value not only in interpreting the different behavior of these two types of plants when grown under severe climatic conditions, but also in helping to gain a better general knowledge of the fundamental physiological processes of agricultural plants. The observations reported in this paper were made upon plants growing at Garden City, Kans., in 1916 and 1917, and at Manhattan, Kans., in 1919.

HISTORICAL

The basis of our knowledge of the carbohydrates of the leaves was laid by Sachs (*12*, *13*, *14*)[3] when he proved that the appearance of starch in the chloroplasts is a direct outcome of the fixation of carbon under the influence of sunlight and chlorophyll. He further stated that starch is the first visible product of carbon assimilation and that it is translocated from the leaves in the form of sugar. Kayser (*8*) found both cane sugar and reducing sugar in the leaves of the beet, grape, potato, and onion and succeeded in separating cane sugar in the crystalline form from the leaves of the grape. Girard (*7*) found that the amount of cane sugar in the leaves of the beet increased during the day, but that the amount of hexoses remained approximately constant. Schimper (*15*) concluded that glucose formation precedes starch formation in the leaves and that starch is formed from glucose when its concentration exceeds a certain maximum, which differs in different plants. Meyer (*9*) observed that certain plants form little or no starch in their leaves and that when starch is found in leaves the amount of sugar present is relatively low, while when starch is absent the sugar content is relatively high. Brown and Morris (*1*) in their classical work on the carbohydrates of the leaves of nasturtium considered that the dextrose and laevulose present in the leaves are more readily accounted for as the products of the hydrolysis of cane sugar than as its precursors. On account of the relative amount of cane sugar found in the leaves and the manner in which it

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[2] The carbohydrate determinations were made by the Department of Chemistry, Kansas Agricultural Experiment Station, the funds for the work being furnished by the Department of Botany. Acknowledgments are due Prof. W. L. Latshaw, under whose supervision the chemical analyses were conducted.

[3] Reference is made by number (italic) to "Literature cited," p. 807-808.

fluctuates during the day, they concluded that it is the first sugar formed in photosynthesis. Strakosch (*17*) studied the sugars in the leaves of the sugar beet by microchemical methods and concluded that dextrose is the first sugar formed in the mesophyll of the leaves of this plant. Parkin (*11*) from his observations on the carbohydrates of the leaves of the snowdrop (*Galanthus nivalis*) expressed the opinion that cane sugar is the first recognizable sugar to appear in the leaves of this plant and that the dextrose and laevulose rise from the inversion of cane sugar. He also found that there is more cane sugar in proportion to reducing sugars in the leaves during the earlier part of the growing season than during the latter part. Davis (*3, 4, 5*) and his coworkers concluded from their investigations of the carbohydrates of the leaves of the mangold and potato that cane sugar is the first sugar formed in the mesophyll of the leaves under the influence of chlorophyll and sunlight. They believed that this cane sugar is transformed into the hexoses in the veins and midribs of the leaves and that it is translocated to the places of storage in that form. They observed, as did Parkin, that in the earlier part of the season cane sugar is present in the leaf tissue in excess of the hexoses, but later in the season the reverse is true. Dixon and Mason (*6*) concluded from microchemical tests that hexoses are the first sugars formed in photosynthesis in the chloroplasts and that the comparatively large amount of cane sugar observed in chemical analyses is only a temporary storage product in the vacuoles of the green cells. Spoehr (*16*) in his observations on the seasonal variation of carbohydrates in *Opuntia phaeacantha* found that a low water content and high temperatures are associated with an increase of polysaccharids, a decrease of monosaccharids, and an increase of pentosans, while a high water content and lower temperatures are associated with a decrease of polysaccharids, an increase of monosaccharids, and a decrease of pentosans. Colin and Belval (*2*) could detect no carbohydrates in the leaves of the wheat plant except cane sugar and its hydrolytic products. Ver Hulst, Peterson and Fred (*18*) found that the pentosans of the leaves of corn approximated 19 per cent of the dry weight of the leaves from the time of tasseling to the dent stage, while the free pentoses amounted to only 0.52 per cent of the dry matter of the leaves during the same period.

EXPERIMENTAL METHODS

CULTURAL METHODS

The plants were grown under field conditions in rows 44 inches apart. The corn plants were thinned to a distance of 2 feet in the row and the sorghum plants to approximately 1 foot. The soil was kept free from weeds by hoeing, but no other cultivation was given. The moisture in the soil at the various periods of leaf sampling is given in Table I.

COLLECTION OF MATERIAL

With but one exception, the experiments extended over a period of 24 hours, and the material for chemical analysis was collected at two-hour intervals during that time. The three uppermost fully unfolded leaves of the plants furnished the material in each case. After the leaves had been stripped from the plants, the distal third and the basal third of each leaf were discarded and the remaining portions, after the midribs had

been removed, were ground in a food chopper. The ground material was immediately placed in three times its volume of 95 per cent ethyl alcohol and sealed in glass jars. The time required to collect the material in this manner at each two-hour period approximated 15 to 20 minutes. At a convenient time the alcoholic material was placed in a hot-air oven and dried for 24 hours at a temperature of 100° to 105° C. The material was then placed in sealed glass jars until the chemical determinations could be made.

TABLE I.—*Moisture content of the soil at the time of leaf sampling in 1916 and 1917 at Garden City, Kans., and in 1919 at Manhattan, Kans.*

Date.	Percentage of moisture at a depth of—					
	1 foot.	2 feet.	3 feet.	4 feet.	5 feet.	6 feet.
Corn plot:						
July 20, 1916	10.0	14.7	19.0	21.9	23.6	24.2
August 1, 1916	7.3	11.8	14.0	18.1	21.4	22.0
Wilting coefficient	12.3	15.9	12.4	15.0	16.3	16.4
Milo plot:						
July 20, 1916	11.1	15.4	19.6	22.2	24.1	24.8
August 1, 1916	8.3	9.9	14.6	14.9	20.8	24.0
Wilting coefficient	12.2	13.4	15.1	14.3	15.4	15.6
Corn plot:						
July 25, 1917	11.2	11.6	18.4	19.3	23.1	22.3
Wilting coefficient	12.6	12.2	15.3	13.9	16.9	17.8
Milo plot:						
July 25, 1917	8.8	18.0	19.2	19.7	21.6	21.6
Wilting coefficient	12.3	15.9	12.4	15.0	16.3	16.4
Corn and sorghum plots:						
July 17, 1919	16.5	21.6	22.1	21.4	.....	.....
Wilting coefficient	12.2	12.5	12.1	12.7	.....	.....

ACTUAL AMOUNT OF CONSTITUENTS

Since the dry weight of a green leaf fluctuates through a considerable range during a 24-hour period, the analyses of leaf material expressed in percentage of dry weight only approximates the changes that occur in the leaf during a given period. The changes in the various constituents of the leaf may be followed more accurately by the determination of the dry weight of a unit of leaf area for each period that analyses are made, and from the percentage data for that period determine the actual amount of the various constituents in any unit of leaf area. The dry weight of a unit of leaf area at the close of each of the two-hour periods during the experiments was determined by the method previously described by the writer (*10*), and the amount of each of the constituents was expressed in grams per square meter of leaf.

CHEMICAL METHODS

The carbohydrates were estimated according to the methods of the Association of Official Agricultural Chemists.[4] In 1916 and 1917 the nonreducing sugars were determined by the difference between the total

[4] ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. As compiled by the Committee on Revision of Methods. Revised to Nov. 1, 1919. 417 p., 18 fig. Washington, D. C. 1920. Bibliographies at ends of chapters.

sugars and the reducing sugars, but in 1919 the nonreducing sugars were estimated as sucrose. In the experiments of 1917 and in the case of corn in 1919, determinations were made of starch according to the official method of direct acid hydrolysis. In this method, however, the material estimated as starch includes the pentosans and other insoluble carbohydrate bodies which undergo hydrolysis and conversion into reducing sugars on boiling with hydrochloric acid. The results, while not indicating the actual amount of starch, do show the increase or decrease of the complex carbohydrates in the leaves during a 24-hour period.

DISCUSSION OF EXPERIMENTAL DATA

Three comparative experiments were conducted with Dwarf Yellow milo and Pride of Saline corn and one with Dwarf Yellow milo and Red Amber sorgo. In one experiment in 1917 Dwarf Yellow milo alone was under observation, while in 1919 in one experiment Pride of Saline corn was the only plant studied. During the three years, observations were thus made upon 10 different sets of plants. The two kinds of plants used in each of the comparative experiments were of the same age and were grown in alternate rows under the same cultural conditions, so that any differences that are observed in the changes of carbohydrates can be attributed to the specific differences of the plants under consideration. A general description of the plants at the time the material was collected is given in Table III. The evaporating power of the air during the time the plants were under observation was measured by Livingston porous cup atmometers, and the evaporation for each two-hour period during the time of each experiment is given in Table II.

TABLE II.—*Evaporation (in cubic centimeters) for the different periods of leaf sampling in 1916 and 1917, at Garden City, Kans., and in 1919, at Manhattan, Kans.*

Date.	Evaporation for period ending—											
	A. M.			P. M.						A. M.		
	8	10	12	2	4	6	8	10	12	2	4	6
1916.												
July 20	4.9	7.5	11.8	14.3	14.6	13.5	8.1	7.1	2.5	1.5	1.0	3.6
August 1	8.5	14.5	17.0	18.1	15.5	12.8	8.7	7.4	5.5	4.5	3.6	5.2
1917.												
July 25	2.9	6.3	8.0	9.3	9.7	8.6	8.5	4.1	2.7	1.3	1.6	0.6
August 3	2.6	7.1	9.3	10.5	11.5	11.6	9.9	.....	.....	.....	.....	.....
1919.												
July 3	.....	6.1	9.8	12.6	15.0	15.5	14.5	8.3	4.0	3.8	2.9	1.8
July 17	.....	1.9	7.6	9.5	9.7	7.8	7.1	4.6	3.1	2.9	1.0	0.6

TABLE III.—*General description of plants used in leaf sampling in 1916 and 1917 at Garden City, Kans., and in 1919 at Manhattan, Kans.*

Date of sampling.	Crop.	Height of plant.	General remarks.
1916.			
July 20–21.....	Corn, Pride of Saline.	3	8 fully unfolded leaves. Visible wilting 11 a. m. to 5 p. m.; guttation showing at 3 a. m.
	Sorghum, Dwarf Yellow milo.	2	Plants booting; no visible wilting; guttation showing first at 1 a. m.
Aug. 1–2.....	Corn, Pride of Saline.	4–5	12–14 leaves. Tassels just showing; leaves wilted from 11 a. m. to 4 p. m.; no guttation during night.
	Sorghum, Dwarf Yellow milo.	4	10–12 leaves. Plants in bloom; leaves slightly wilted from 1–3 p. m.; no guttation during night.
1917.			
July 25–26.....	Corn, Pride of Saline.	4	10 fully unfolded leaves; leaves not wilted during day; guttation showing at 3 a. m.
	Sorghum, Dwarf Yellow milo.	2	10 fully unfolded leaves; leaf of boot showing. No wilting during the day; guttation heavy from midnight until morning.
Aug. 3.....	Sorghum, Dwarf Yellow milo.	2–3	"Booting." No wilting of leaves during the day.
July 3–4.....	Corn, Pride of Saline.	5	9 fully unfolded leaves. Leaves not visibly wilted during the day; guttation heavy after 2 a. m.
July 17–18....	Sorghum, Dwarf Yellow milo.	3	8 fully unfolded leaves. No signs of wilting during day; heavy guttation after 1 a. m.
	Sorghum, Red Amber.	5	9 fully unfolded leaves. No wilting; heavy guttation after 1 a. m.

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TABLE IV.—*Daily variation of water, dry matter and carbohydrates in the leaves of Pride of Saline corn and Dwarf milo at Garden City, Kans., July 20 and 21, 1916*

PERCENTAGE OF THE CONSTITUENTS

Period ending—	Dry matter.		Water.		Total sugars.		Nonreducing sugars.		Reducing sugars.	
	Corn.	Milo.	Corn.	Milo.	Corn.	Milo.	Corn.	Milo.	Corn.	Milo.
July 20.										
6 a. m.	26.3	29.6	73.7	70.4	2.75	3.68	1.87	2.44	0.88	1.24
8 a. m.	27.3	30.6	72.7	69.4	4.68	4.64	3.44	3.19	1.24	1.45
10 a. m.	28.1	31.3	71.9	68.7	5.13	5.24	4.01	3.94	1.13	1.30
12 m.	28.7	32.4	71.3	67.6	4.89	5.50	4.25	4.80	.64	.70
2 p. m.	29.4	33.0	70.6	67.0	6.03	8.27	4.83	6.98	1.20	1.29
4 p. m.	28.9	33.6	71.1	66.4	5.13	.....	4.44	.....	.69	.....
6 p. m.	28.1	32.8	71.9	67.2	4.58	6.55	3.65	5.25	.93	1.30
8 p. m.	26.8	31.5	73.2	68.5	3.31	4.22	2.54	3.28	.77	.94
10 p. m.	26.3	30.3	73.7	69.7	2.33	2.89	1.95	2.35	.38	.54
12 midn.	25.4	30.0	74.6	70.0	1.86	2.82	1.51	2.22	.35	.60
July 21.										
2 a. m.	24.3	28.4	75.7	71.6	2.19	3.16	1.60	2.20	.59	.96
4 a. m.	24.5	29.1	75.5	71.9	1.73	3.44	1.11	2.09	.62	1.35
6 a. m.	26.4	29.3	73.6	70.7	2.61	3.75	2.02	2.52	.59	1.23

GRAMS OF CONSTITUENTS PER SQUARE METER OF LEAF

July 20.										
6. a. m.	44.0	46.6	123.7	111.3	1.21	1.71	0.82	1.14	0.39	0.57
8 a. m.	45.3	47.2	120.7	107.0	2.12	2.18	1.56	1.51	.56	.67
10 a. m.	46.7	48.5	119.5	106.5	2.39	2.54	1.87	1.91	.52	.63
12 m.	47.3	49.9	117.7	104.5	2.31	2.74	2.01	2.39	.30	.35
2 p. m.	48.8	52.5	117.3	106.7	2.94	4.34	2.36	3.66	.59	.68
4 p. m.	48.5	54.2	119.5	107.2	2.49	.....	2.15	.....	.34	.....
6 p. m.	47.5	52.8	121.8	108.7	2.17	3.46	1.73	2.77	.44	.69
8 p. m.	46.4	52.0	127.3	113.4	1.34	2.19	1.03	1.71	.31	.48
10 p. m.	45.0	50.3	126.7	116.2	1.05	1.45	.88	1.18	.17	.27
12 midn.	43.0	49.7	126.5	116.1	.80	1.40	.65	1.10	.15	.30
July 21.										
2 a. m.	41.2	47.9	128.9	120.9	.90	1.51	.66	1.05	.24	.46
4 a. m.	41.7	46.7	128.7	119.5	.72	1.61	.46	.98	.26	.63
6 a. m.	44.2	48.1	123.5	116.5	1.15	1.80	.89	1.21	.26	.59

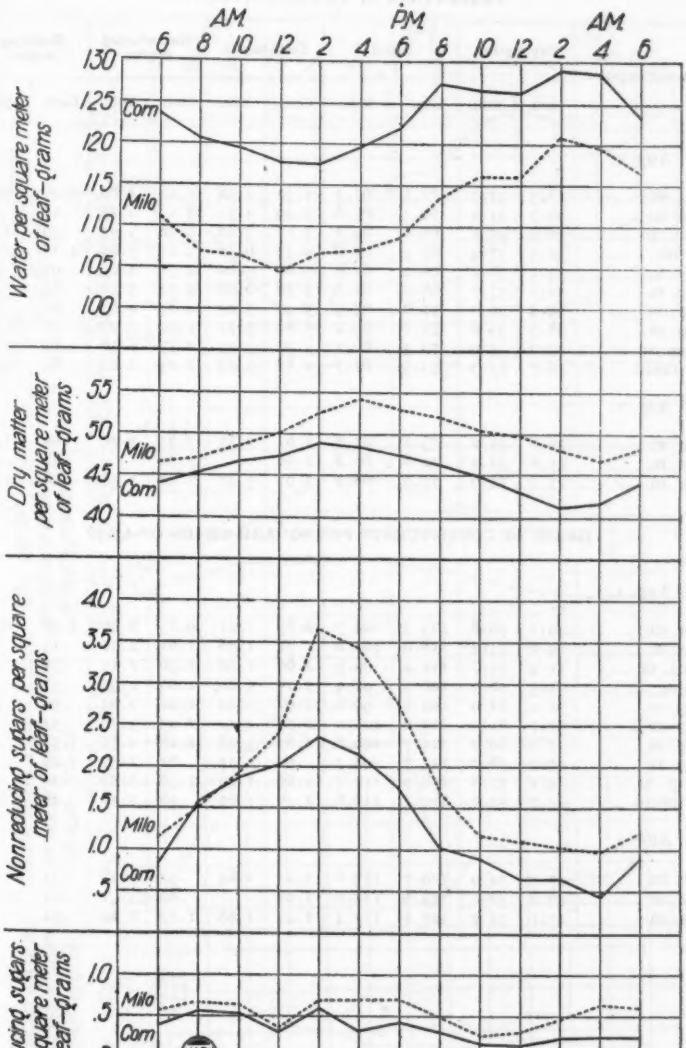

FIG. 1.—Graph showing the variation of the reducing and nonreducing sugars, dry matter, and water in the leaves of Pride of Saline corn and Dwarf Yellow milo during July 20 and 21, 1916.

TABLE V.—*Daily variation of water, dry matter, and carbohydrates in the leaves of Pride of Saline corn and Dwarf milo at Garden City, Kans., August 1 and 2, 1916*

PERCENTAGE OF THE CONSTITUENTS

Period ending—	Dry matter.		Water.		Total sugars.		Nonreducing sugars.		Reducing sugars.	
	Corn.	Milo.	Corn.	Milo.	Corn.	Milo.	Corn.	Milo.	Corn.	Milo.
Aug. 1.										
6 a. m.	28.5	32.7	71.5	67.3	1.52	2.38	1.12	1.89	0.40	0.49
8 a. m.	29.7	34.4	70.3	65.6	3.49	3.33	3.23	2.45	.26	.88
10 a. m.	31.4	35.8	68.6	64.2	5.17	5.20	4.58	3.93	.59	1.27
12 m.	32.5	37.4	67.5	62.6	6.33	6.52	5.21	5.09	1.12	1.43
2 p. m.	33.1	38.2	66.9	61.8	5.68	5.62	4.71	4.65	.97	.97
4 p. m.	31.1	37.7	68.9	62.3	5.38	6.68	4.74	5.14	.64	1.54
6 p. m.	29.4	36.1	70.6	63.9	5.49	5.45	4.87	4.63	.62	.82
8 p. m.	28.9	34.8	71.1	65.2	1.80	3.71	1.22	2.70	.58	1.01
10 p. m.	27.7	33.9	72.3	66.1	3.39	3.39	2.77	2.58	.62	.81
12 midn.	26.7	32.3	73.3	67.7	2.88	3.24	2.03	2.02	.85	1.22
Aug. 2.										
2 a. m.	26.8	32.2	73.2	67.8	2.64	3.35	1.95	2.20	.69	1.15
4 a. m.	27.2	31.2	72.8	68.8	2.30	.....	1.72	.....	.58	.....
6 a. m.	27.1	32.6	72.9	68.4	3.01	3.47	2.33	2.38	.68	1.09

GRAMS OF CONSTITUENTS PER SQUARE METER OF LEAF

Aug. 1.										
6 a. m.	49.1	50.8	123.3	104.7	0.75	1.21	0.55	0.96	0.20	0.25
8 a. m.	50.0	53.4	118.6	102.0	1.74	1.78	1.61	1.31	.13	.47
10 a. m.	51.4	55.7	112.4	99.9	2.66	2.90	2.36	2.19	.30	.71
12 m.	51.3	58.3	106.9	97.9	3.25	3.80	2.68	2.97	.57	.83
2 p. m.	51.4	61.0	104.2	99.0	2.92	3.43	2.42	2.84	.50	.59
4 p. m.	51.7	61.5	115.0	102.0	2.78	4.11	2.45	3.16	.33	.95
6 p. m.	51.0	60.2	122.7	106.6	2.80	3.28	2.48	2.79	.32	.49
8 p. m.	50.2	58.0	123.7	108.7	.90	2.15	.61	1.57	.29	.58
10 p. m.	49.1	57.8	128.6	112.7	1.66	1.96	1.36	1.49	.30	.47
12 midn.	47.7	55.6	131.1	116.6	1.37	1.80	.97	1.12	.40	.68
Aug. 2.										
2 a. m.	47.3	54.9	129.2	115.6	1.25	1.84	.92	1.21	.33	.63
4 a. m.	46.6	52.1	124.9	115.0	1.07	.....	.80	.....	.27	.....
6 a. m.	47.1	54.2	127.2	117.4	1.42	1.88	1.10	1.29	.32	.59

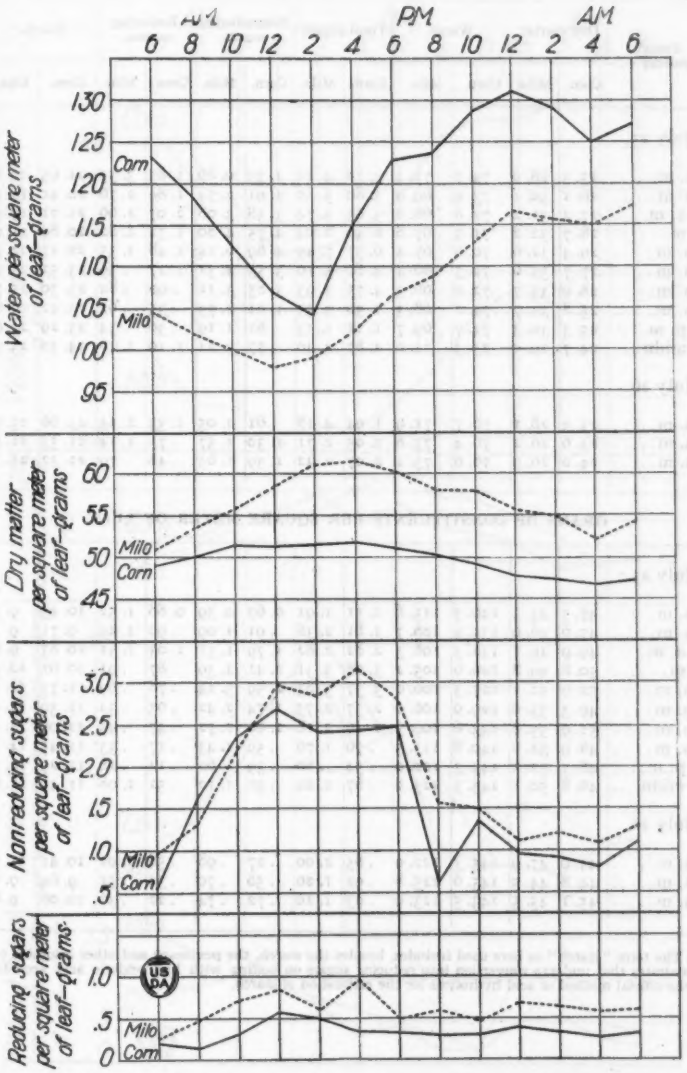

FIG. 2.—Graph showing the variation of the reducing and nonreducing sugars, dry matter, and water in the leaves of Pride of Saline corn and Dwarf Yellow milo during August 1 and 2, 1916.

TABLE VI.—*Daily variation of water, dry matter, and carbohydrates in the leaves of Pride of Saline corn and Dwarf milo at Garden City, Kans., July 25 and 26, 1917*

PERCENTAGE OF THE CONSTITUENTS

Period ending—	Dry matter.		Water.		Total sugars.		Nonreducing sugars.		Reducing sugars.		Starch.[a]	
	Corn.	Milo.	Corn.	Milo.	Corn.	Milo.	Corn.	Milo.	Corn.	Milo.	Corn.	Milo.
July 25.												
6 a. m.	25.3	28.6	74.7	71.4	3.18	4.21	1.33	0.86	1.85	3.35	21.95	20.67
8 a. m.	26.1	30.1	73.9	69.9	3.80	5.10	1.91	2.34	1.89	2.76	20.41	19.54
10 a. m.	27.4	31.2	72.6	68.8	5.65	5.74	3.58	3.08	2.07	2.66	21.71	19.98
12 m.	28.7	32.2	71.3	67.8	6.46	6.64	4.75	4.80	1.71	1.82	20.89	21.07
2 p. m.	29.4	32.6	70.6	67.4	6.37	7.49	4.89	6.14	1.48	1.35	22.17	22.98
4 p. m.	27.7	33.6	72.3	66.4	4.80	5.10	3.53	4.51	1.27	.59	23.51	23.29
6 p. m.	28.0	33.7	72.0	66.3	4.75	3.93	3.85	3.11	.90	.82	23.30	25.70
8 p. m.	25.8	31.7	74.2	68.3	1.56	3.35	1.21	2.73	.35	.62	23.42	26.82
10 p. m.	25.3	30.3	74.7	69.7	1.11	1.53	.81	1.19	.30	.34	23.29	23.63
12 midn.	24.7	29.0	75.3	71.0	1.87	4.40	.77	2.24	1.10	2.16	24.50	25.34
July 26.												
2 a. m.	23.3	28.1	76.7	71.9	1.94	4.18	.61	2.05	1.33	2.13	23.66	23.51
4 a. m.	23.6	26.2	76.4	73.8	2.05	2.71	1.30	1.57	.75	1.14	21.53	21.55
6 a. m.	24.0	26.9	76.0	73.1	2.07	2.42	1.59	1.63	.48	.79	22.32	21.16

GRAMS OF CONSTITUENTS PER SQUARE METER OF LEAF

July 25.												
6 a. m.	47.5	45.3	140.5	113.6	1.51	1.91	0.63	0.39	0.88	1.52	10.43	9.36
8 a. m.	47.6	46.6	135.4	108.7	1.81	2.38	.91	1.09	.90	1.29	9.71	9.10
10 a. m.	49.9	49.1	132.3	108.3	2.82	2.82	1.79	1.51	1.03	1.31	10.83	9.81
12 m.	50.8	49.8	126.6	105.2	3.28	3.31	2.41	2.39	.87	.91	10.61	10.49
2 p. m.	52.9	51.1	127.3	106.0	3.37	3.83	2.59	3.14	.78	.69	11.73	11.74
4 p. m.	49.3	53.6	129.0	106.0	2.37	2.73	1.74	2.42	.63	.32	11.59	12.48
6 p. m.	51.9	55.4	133.6	109.3	2.46	2.18	2.00	1.72	.47	.45	12.09	14.24
8 p. m.	48.9	52.6	140.8	113.5	.76	1.76	.59	1.43	.17	.33	11.45	14.11
10 p. m.	48.5	52.1	143.3	120.0	.54	.80	.39	.62	.14	.18	11.29	12.31
12 midn.	46.8	50.1	143.3	123.0	.87	2.20	.36	1.12	.51	1.08	11.47	12.69
July 26.												
2 a. m.	44.0	47.9	145.3	122.9	.85	2.00	.27	.98	.58	1.02	10.41	11.26
4 a. m.	44.8	44.5	145.6	125.8	.92	1.20	.58	.70	.34	.51	9.64	9.59
6 a. m.	45.1	45.5	143.5	123.9	.93	1.10	.72	.74	.22	.36	10.06	9.63

[a] The term "starch" as here used includes, besides the starch, the pentosans and other insoluble carbohydrates that undergo conversion into reducing sugars on boiling with hydrochloric acid according to the official method of acid hydrolysis for the estimation of starch.

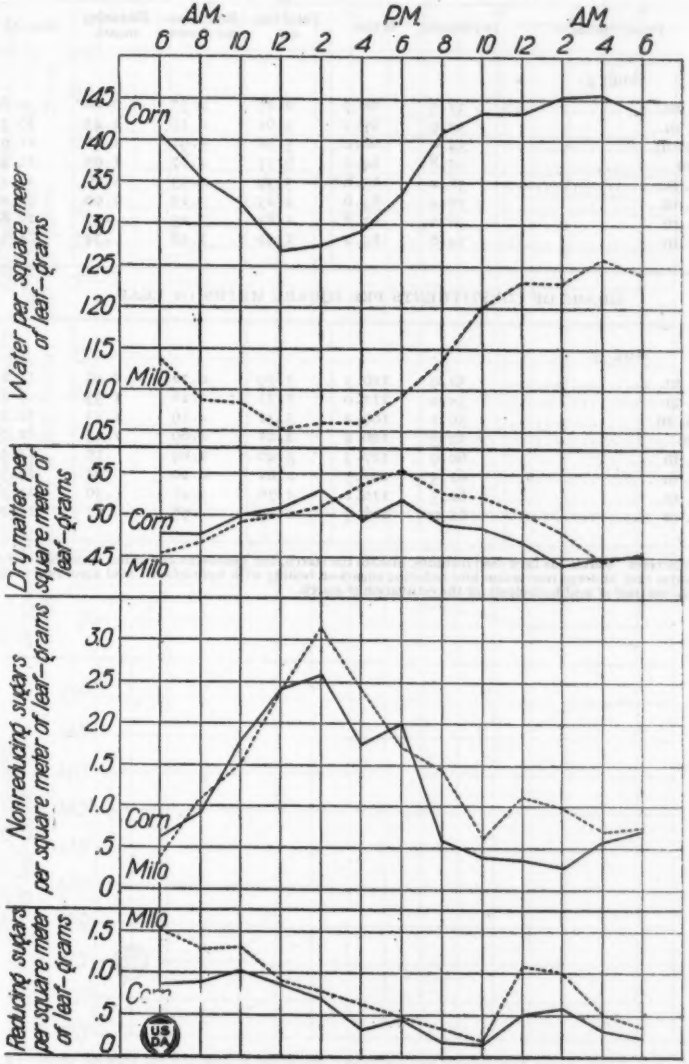

FIG. 3.—Graph showing the variation of the reducing and nonreducing sugars, dry matter and water in the leaves of Pride of Saline corn and Dwarf Yellow milo during July 25 and 26, 1917.

TABLE VII.—*Daily variation of water, dry matter and carbohydrates in the leaves of Dwarf milo at Garden City, Kans., August 3, 1917*

PERCENTAGE OF THE CONSTITUENTS

Period ending—	Dry matter.	Water.	Total sugars.	Nonreducing sugars.	Reducing sugars.	Starch.[a]
Aug. 3.						
6 a. m.	31.3	68.7	2.25	1.37	0.88	20.86
8 a. m.	32.9	67.1	4.62	2.17	2.45	19.51
10 a. m.	34.0	66.0	5.92	3.91	2.01	21.91
12 m.	35.8	64.2	6.77	4.87	1.90	21.44
2 p. m.	36.2	63.8	5.59	4.33	1.26	22.23
4 p. m.	36.4	63.6	4.25	3.25	1.00	23.29
6 p. m.	36.2	63.8	2.82	2.25	.57	23.84
8 p. m.	34.8	65.2	1.92	1.58	.34	24.23

GRAMS OF CONSTITUENTS PER SQUARE METER OF LEAF

Aug. 3.						
6 a. m.	52.9	116.3	1.19	0.72	0.46	11.03
8 a. m.	54.4	111.0	2.51	1.18	1.33	10.61
10 a. m.	56.1	109.2	3.32	2.19	1.13	12.29
12 m.	59.3	106.4	4.01	2.89	1.13	12.79
2 p. m.	60.9	107.5	3.40	2.64	.76	13.54
4 p. m.	61.5	107.5	2.61	2.00	.61	14.32
6 p. m.	62.5	110.2	1.76	1.41	.36	14.90
8 p. m.	61.9	116.3	1.19	.98	.21	15.00

[a] The term "starch" as here used includes, besides the starch, the pentosans and other insoluble carbohydrates that undergo conversion into reducing sugars on boiling with hydrochloric acid according to the official method of acid hydrolysis for the estimation of starch.

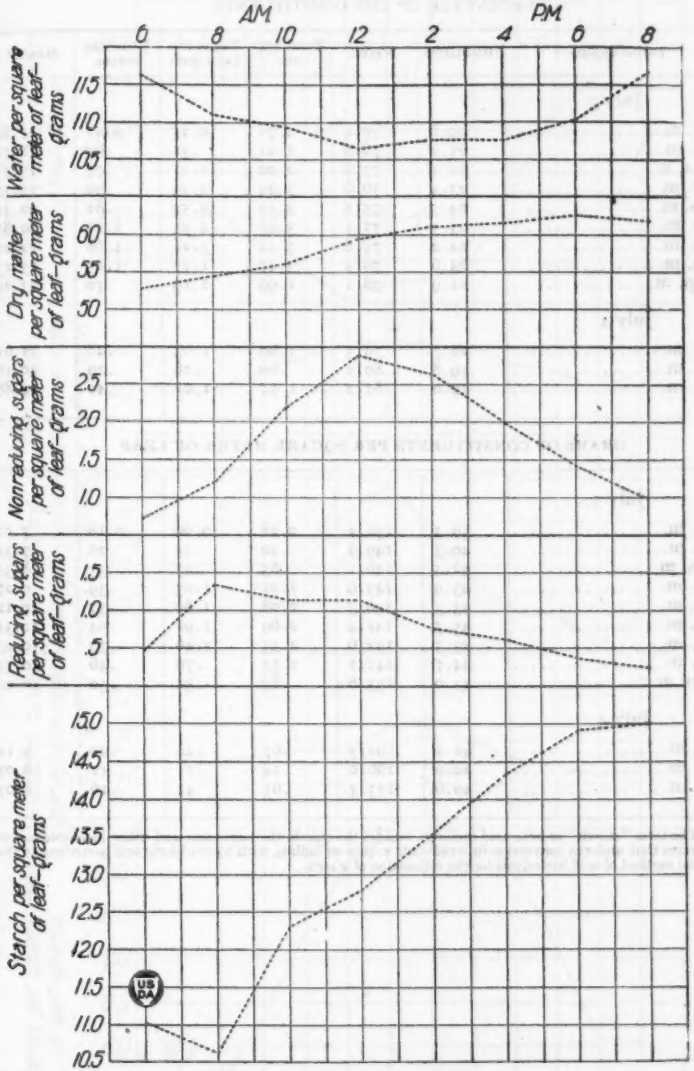

FIG. 4.—Graph showing the variation of the starch,[5] reducing and nonreducing sugars, dry matter, and water in the leaves of Dwarf Yellow milo during August 3, 1917.

[5] The term "starch" as here used includes, besides the starch, the pentosans and other insoluble carbohydrates that undergo conversion into reducing sugars on boiling with hydrochloric acid according to the official method of acid hydrolysis for the estimation of starch.

TABLE VIII.—*Daily variation of water, dry matter, and carbohydrates in the leaves of Pride of Saline corn, at Manhattan, Kans., July 3 and 4, 1919*

PERCENTAGE OF THE CONSTITUENTS

Period ending—	Dry matter.	Water.	Total sugars.	Nonreducing sugars.	Reducing sugars.	Starch.[a]
July 3.						
7 a. m.	20.6	79.4	0.71	0.25	0.42	17.84
9 a. m.	21.2	78.8	1.01	.31	.65	18.72
11 a. m.	22.5	77.5	2.00	1.11	.79	19.75
1 p. m.	23.4	76.6	3.44	2.34	.90	21.12
3 p. m.	24.5	75.5	4.59	3.54	.81	22.34
5 p. m.	24.3	75.7	5.92	4.23	1.41	22.85
7 p. m.	24.2	75.8	5.14	3.22	1.68	22.22
9 p. m.	23.6	76.4	3.47	1.77	1.05	23.13
11 p. m.	21.9	78.1	1.99	1.19	.70	23.29
July 4.						
1 a. m.	20.7	79.3	1.60	1.04	.48	21.61
3 a. m.	19.8	80.2	.60	.28	.29	20.18
5 a. m.	19.0	81.0	1.51	1.02	.40	19.88

GRAMS OF CONSTITUENTS PER SQUARE METER OF LEAF

July 3.						
7 a. m.	39.3	152.2	0.28	0.09	0.16	7.01
9 a. m.	40.3	149.9	.40	.12	.26	7.54
11 a. m.	42.3	146.1	.85	.47	.33	8.35
1 p. m.	43.9	143.9	1.51	1.03	.39	9.27
3 p. m.	45.3	140.3	2.08	1.60	.37	10.12
5 p. m.	45.5	142.4	2.69	1.92	.64	10.34
7 p. m.	45.3	142.6	2.33	1.46	.76	10.07
9 p. m.	44.7	145.2	1.55	.79	.46	10.33
11 p. m.	43.0	153.8	.85	.51	.30	10.01
July 4.						
1 a. m.	42.2	162.5	.67	.44	.20	9.12
3 a. m.	40.0	166.0	.24	.11	.12	8.07
5 a. m.	40.6	173.3	.61	.41	.16	8.07

[a] The term "starch" as here used includes, besides the starch, the pentosans and other insoluble carbohydrates that undergo conversion into reducing sugars on boiling with hydrochloric acid according to the official method of acid hydrolysis for the estimation of starch.

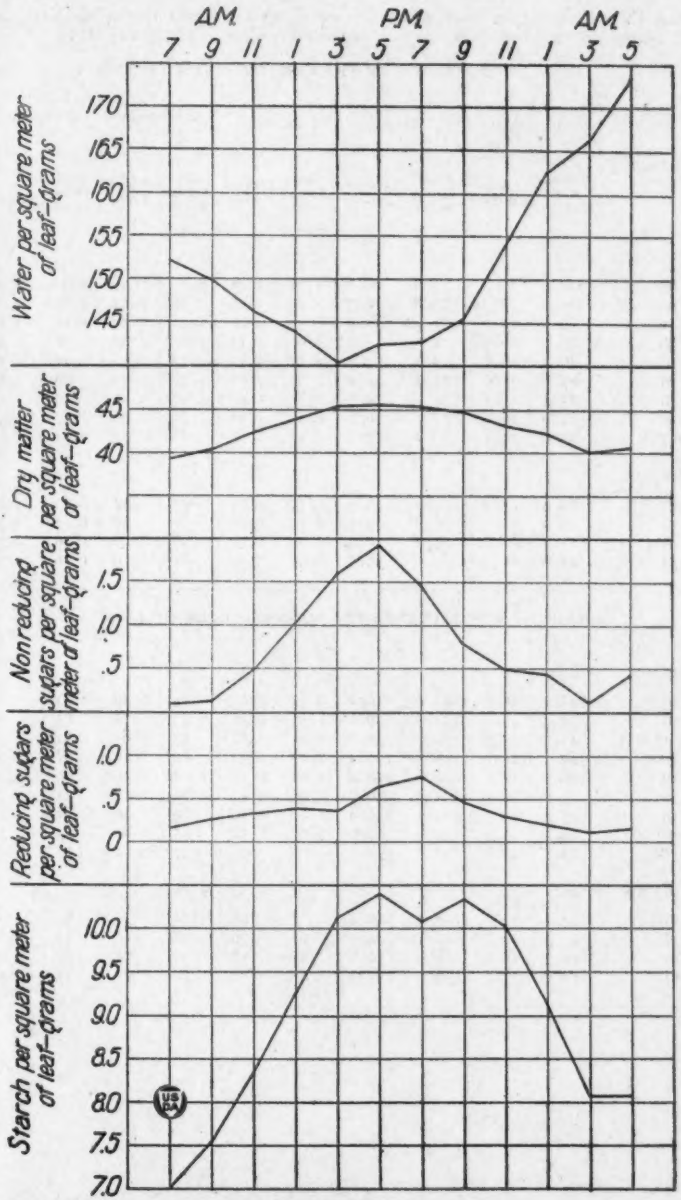

FIG. 5.—Graph showing the variation of the starch,[8] reducing and nonreducing sugars, dry matter, and water in the leaves of Pride of Saline corn during July 3 and 4, 1919.

[8] The term "starch" as here used includes, besides the starch, the pentosans and other insoluble carbohydrates that undergo conversion into reducing sugars on boiling with hydrochloric acid according to the official method of acid hydrolysis for the estimation of starch.

TABLE IX.—*Daily variation of water, dry matter, and carbohydrates in the leaves of Dwarf milo and Red Amber sorgo at Manhattan, Kans., July 17 and 18, 1919*

PERCENTAGE OF THE CONSTITUENTS

Period ending—	Dry matter.		Water.		Total sugars.		Nonreducing sugars.		Reducing sugars.	
	Red Amber sorgo.	Dwarf milo.	Red Amber sorgo.	Dwarf milo.	Red Amber sorgo	Dwarf milo.	Red Amber sorgo.	Dwarf milo.	Red Amber sorgo.	Dwarf milo.
July 17.										
7 a. m.	22.4	21.4	77.6	78.6	1.74	2.28	0.83	0.76	0.82	1.41
9 a. m.	24.2	23.3	75.8	76.7	2.53	3.16	.88	1.41	1.53	1.61
11 a. m.	24.6	25.0	75.4	75.0	3.34	4.20	1.98	2.18	1.18	1.84
1 p. m.	26.0	26.0	74.0	74.0	4.06	5.17	1.85	2.82	2.02	2.01
3 p. m.	26.4	26.7	73.6	73.3	4.11	5.25	2.43	2.50	1.48	2.53
5 p. m.	25.6	25.9	74.4	74.1	2.17	4.88	1.38	2.36	.68	2.30
7 p. m.	25.3	25.5	74.7	74.5	2.81	5.49	1.97	3.30	.70	1.94
9 p. m.	24.9	24.0	75.1	76.0	2.33	3.57	1.25	2.06	.97	1.34
11 p. m.	25.0	24.5	75.0	75.5	2.13	2.13	.82	.78	1.21	1.25
July 18.										
1 a. m.	24.4	23.5	75.6	76.5	1.77	2.05	.28	.72	1.41	1.22
3 a. m.	23.4	22.9	76.6	77.1	2.13	1.86	.79	.48	1.24	1.29
5 a. m.	22.2	21.6	77.8	78.4	2.14	1.38	.86	.16	1.16	1.15
7 a. m.	21.0	20.5	79.0	79.5	2.07	1.32	.60	.16	1.38	1.10

GRAMS OF CONSTITUENTS PER SQUARE METER OF LEAF

July 17.										
7 a. m.	41.1	40.6	143.0	150.0	0.71	0.93	0.34	0.40	0.34	0.57
9 a. m.	42.4	41.2	133.5	136.0	1.07	1.30	.37	.58	.65	.66
11 a. m.	43.0	43.4	132.0	130.6	1.44	1.82	.85	.95	.51	.80
1 p. m.	45.2	44.6	128.7	127.1	1.83	2.30	.84	1.25	.91	.90
3 p. m.	46.5	46.6	129.8	128.4	1.91	2.45	1.13	1.16	.69	1.19
5 p. m.	45.7	45.5	133.3	130.6	.99	2.22	.63	1.07	.31	1.05
7 p. m.	45.4	45.3	134.3	132.4	1.27	2.49	.89	1.49	.32	.88
9 p. m.	44.4	43.1	134.6	139.9	1.03	1.54	.55	.89	.43	.58
11 p. m.	45.3	43.8	136.3	135.5	.96	.93	.37	.34	.55	.55
July 18.										
1 a. m.	44.4	43.0	138.2	140.4	.78	.88	.12	.31	.63	.52
3 a. m.	43.3	42.6	142.1	144.2	.92	.79	.34	.20	.54	.55
5 a. m.	42.3	41.3	148.9	150.1	.91	.57	.36	.07	.49	.47
7 a. m.	40.0	39.0	150.9	151.3	.83	.51	.24	.06	.55	.43

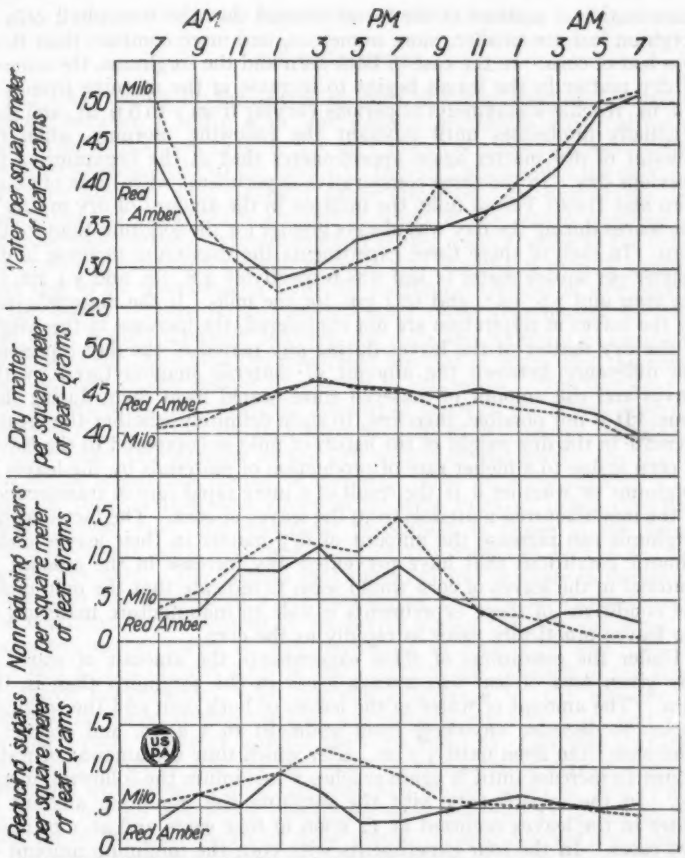

FIG. 6.—Graph showing the variation of the reducing and nonreducing sugars, dry matter, and water in the leaves of Dwarf Yellow milo and Red Amber sorgo during July 17 and 18, 1919.

The data in regard to the amount of water, dry matter, and carbohydrates obtained in each of the experiments are shown in Tables IV to IX. The results are expressed in percentages and in terms of the number of grams of constituents per square meter of leaf. The percentages of water and dry matter are calculated on a wet basis, while the percentages of carbohydrates are estimated on the amount of dry matter in the leaf. The changes in the amount of the constituents per square meter of leaf during each two-hour period of the experiments are shown by graphs in figures 1 to 6.

DRY MATTER AND WATER IN THE LEAVES

The daily variation of the water and dry matter in the leaves of corn and the sorghums has been reported in detail in a previous paper (*10*), so that only those facts will be mentioned that are essential to discussion of the variation of the carbohydrates of the leaves.

In the experiments the amount of dry matter in a given area of leaf was always greater for the sorghums than for the corn, and a microscopic

examination of sections of the leaves showed that the mesophyll cells of sorghum leaf are smaller, more numerous, and more compact than those of a leaf of corn. In the case of both corn and the sorghums, the amount of dry matter in the leaves begins to increase in the morning from 4 to 6 a. m., reaches a maximum at periods varying from 2 to 6 p. m., and then gradually diminishes until daylight the following morning, when the amount of dry matter again approximates that at the beginning of the previous day. In the three comparative experiments with Pride of Saline corn and Dwarf Yellow milo, the increase in the amount of dry matter in the leaves during the day was always greater for the sorghum than for the corn. In each of these three experiments the maximum increase in dry matter per square meter of leaf was respectively 4.8, 2.6, and 5.4 gm. for the corn and 7.6, 10.7, and 10.1 gm. for the milo. If the materials used by the leaves in respiration are not considered, the increase in the weight of the dry matter of the leaves during any period of the day represents the difference between the amount of material manufactured by the leaves and the amount of material translocated from them during that time. It is not possible, therefore, to state definitely whether the greater increase in the dry weight of the leaves of milo as compared to the leaves of corn is due to a higher rate of production of materials by the leaves of sorghums or whether it is the result of a more rapid rate of translocation of the manufactured materials from the leaves of corn. The fact that the sorghums can increase the amount of dry matter in their leaves under climatic conditions that have prevented any increase in the amount of material in the leaves of corn would seem to indicate that the milo under the conditions of these experiments is able to manufacture materials in the leaves practically twice as rapidly as the corn.

Under the conditions of these experiments the amount of water in any given area of leaf was always lower in the sorghums than in the corn. The amount of water in the leaves of both corn and the sorghum begins to decrease anywhere from midnight to 5 a. m. and reaches a minimum from noon until 3 p. m., after which time the amount of water begins to increase until it again reaches a maximum the following morning. In the experiments with the sorghum the minimum amount of water in the leaves occurred at 12 noon in four cases and at 1 p. m. in two cases. In the four experiments with corn the minimum amount of water in the leaves occurred three times at 2 p. m. and once at 3 p. m. In only one case did the quantity of water in the leaves appear to be the limiting factor in the production of dry matter. In the experiment on August 1, 1916, the amount of dry matter in the leaves of corn reached a maximum at 10 a. m. and remained constant until after 4 p. m., when it began to decrease. The amount of water in the leaves at 10 a. m. was 112.4 gm. per square meter of leaf, and it continued to decrease until it amounted to only 104.2 gm. per square meter of leaf at 2 p. m. In none of the other experiments with corn did the amount of water in the leaves reach a minimum lower than 117 gm. per square meter of leaf. In all of the other experiments the amount of dry matter in the leaves of corn continued steadily to increase past the time of the minimum water content of the leaves, so that it would appear that the minimum amount of water that can be reached in the leaves of corn and not interfere with the process of photosynthesis is slightly above 112 gm. per square meter of leaf. The minimum amount of water observed in the leaves of Dwarf Yellow milo and Red Amber sorgo in these experiments was 98 gm. per square meter of leaf, but this minimum apparently in no way retarded the production of dry matter.

THE CARBOHYDRATES OF THE LEAVES

THE TOTAL SUGARS

The total sugars in the leaves of corn and the sorghums begin to increase between 4 and 6 a. m. and reach a maximum which varied in these experiments from 12 m. to 5 p. m. After the maximum was reached the sugar disappeared rapidly until 9 to 10 p. m., after which the decrease was very gradual until daylight again appeared. In five of the ten observations the maximum amount of sugar in the leaves occurred at the same time as the maximum amount of dry matter, while in the other five cases the maximum amount of sugar was reached at periods varying from one to six hours earlier than the maximum amount of dry matter in the leaves. There is no evidence from these experiments to show that there is any difference between the corn and sorghum in regard to the time of day at which the maximum amount of sugar and the maximum amount of dry matter may occur in the leaves. The fact that in some experiments the maximum amount of sugar in the leaves occurred earlier in the day than the maximum amount of dry matter shows that in these cases the increase in the dry weight of the leaves was due in part to the temporary storage of materials other than the sugars.

In the three comparative experiments with Pride of Saline corn and Dwarf Yellow milo, the maximum percentage of sugar in the leaves was respectively 6.03, 6.33, and 6.44 for the corn and 8.27, 6.68, and 7.49 for the milo. The average maximum amount of sugar in the leaves of the milo was thus about 1 per cent higher than in the leaves of corn. The maximum sugar content of the leaves during the day, expressed in grams per square meter of leaf, amounted to 2.94, 3.25, and 3.37 for corn and 4.34, 4.11, and 3.83 for milo, respectively, in each of the three successive experiments. The maximum increase in the amount of sugar in the leaves during the day was respectively 1.73, 2.50, and 1.86 gm. per square meter of leaf for the corn and 2.63, 2.90, and 1.92 gm. per square meter of leaf for the milo. A comparative experiment was conducted with Dwarf Yellow milo and Red Amber sorgo on July 17 and 18, 1919, but no significant differences were observed in regard to the behavior of the total sugars in these plants. Table X gives a summary of the more important facts concerning the changes in sugars of the leaves during the day.

THE INSOLUBLE CARBOHYDRATES

In the experiment of July 25 and 26, 1917, the maximum amount of insoluble carbohydrates per square meter of leaf was reached at 6 p. m. in both the corn and milo, while the maximum sugar content occurred at 2 p. m. In the experiment with Dwarf milo on August 3, 1917, the maximum amount of sugar per square meter of leaf was reached at 12 m., but the maximum amount of insoluble carbohydrates did not occur until 8 p. m. In the observation on corn during the experiment of July 3 and 4, 1919, the maximum amount of sugar and insoluble carbohydrates per square meter of leaf was reached at 5 p. m. After the insoluble carbohydrates reached a maximum in the leaves, they showed little or no diminution in amount until about midnight, when they decreased rapidly until daylight.

TABLE X.—*Summary of the more important facts concerning the changes in the sugar in the leaves of corn and the sorghums during the day*

Date.	Plants.	Maximum amount of total sugars.		Maximum increase in the amount of—							
				Total sugars.		Nonreducing sugars.		Reducing sugars.		Dry matter.	
1916.		*Per cent.*	*Gm.*[2] [a]	*Per cent.*	*Gm.*[2]	*Per cent.*	*Gm.*[2]	*Per cent.*	*Gm.*[2]	*Per cent.*	*Gm.*[2]
July 20–21	Corn, Pride of Saline	6.03	2.94	3.28	1.73	2.96	1.54	0.36	0.20	3.1	4.8
	Sorghum, Dwarf Yellow milo	8.27	4.34	4.59	2.63	4.54	2.52	.21	.12	4.0	7.6
Aug. 1–2	Corn, Pride of Saline	6.33	3.25	4.81	2.50	4.09	2.13	.72	.37	4.0	2.6
	Sorghum, Dwarf Yellow milo	6.68	4.11	4.30	2.90	3.20	2.01	1.05	.70	5.5	10.7
1917.											
July 25–26	Corn, Pride of Saline	6.46	3.37	3.28	1.86	3.56	1.96	.22	.18	4.1	5.4
	Sorghum, Dwarf Yellow milo	7.49	3.83	3.28	1.92	5.28	2.75	None.	None.	5.1	10.1
Aug. 3	Sorghum, Dwarf Yellow milo	6.77	4.01	4.52	2.82	3.50	2.17	1.57	.87	5.1	9.6
1919.											
July 3–4	Corn, Pride of Saline	5.92	2.69	5.21	2.41	3.98	1.83	1.26	.60	3.9	6.2
July 17–18	Sorghum, Dwarf Yellow milo	5.49	2.49	3.21	1.56	2.54	1.09	1.02	.62	5.3	6.0
	Sorghum, Red Amber	5.06	1.91	2.37	1.20	1.60	.79	1.10	.57	4.0	5.4

[a] The abbreviation Gm.[2] is used to express the number of grams of the constituents per square meter of leaf.

The maximum increase in the dry matter of the leaves of corn and milo was always greater than the increase of the carbohydrates as determined by the methods used in these experiments. The case of Dwarf Yellow milo in the experiment of July 25, 1917, will serve as a concrete example of the above statement. On that date at 6 a. m. the dry matter in a square meter of leaf amounted to 45.3 gm. This increased gradually during the day until a maximum was reached at 6 p. m., when the dry matter amounted to 55.4 gm. per square meter of leaf. Thus each square meter of leaf increased in dry weight 10.1 gm. during the 12-hour period. The increase in sugars and insoluble carbohydrates from 6 a. m. to 6 p. m. amounted to 0.27 and 4.88 gm. per square meter of leaf, respectively, so that the total increase in dry weight in each square meter of leaf due to the carbohydrates amounted to 5.15 gm. There was thus an increase of 4.95 gm. of dry matter in each square meter of leaf that could not be accounted for by the increase of carbohydrates. In the several experiments the increase of dry matter during the day that could not be accounted for by the increase in sugar and insoluble carbohydrates during the same period varied from 7.4 to 53.7 per cent of the total increase of dry matter. Detailed information on this point can be obtained by consulting Tables VI, VII, and VIII. The undetermined material is evidently not protein, since uupublished data show that in the cases in question there is no increase in the absolute amount of nitrogen in the leaves during the day. The method used in obtaining the dry weight of a unit of area of leaf does not seem to be at fault, since the dry weight of a unit of area in all the experiments was approximately the same at the close of a 24-hour period as it was at the beginning. Since the samples used in determining the weight of a unit of leaf area at the beginning and at the close of an experiment were selected from rather widely separated portions of the leaf, it would seem that the structure of the leaf was uniform and that no appreciable error would be due to variations in the leaf samples that were used to determine the absolute amount of dry matter. The discrepancy observed between the total increase in dry matter in the leaves during the day and the increase in carbohydrates is due apparently to the fact that certain temporary storage products in the leaves are not detected by the methods used in determining the carbohydrates.

THE REDUCING AND NONREDUCING SUGARS

The nonreducing sugars in the leaves of the plants studied were, with the exception of the experiment with Dwarf milo and Red Amber sorgo, always in excess of the reducing sugars. The increase in the amount of the nonreducing sugars and the maximum point of increase of the total sugars is also the maximum point of increase of the nonreducing sugars. In the three comparative experiments with Pride of Saline corn and Dwarf Yellow milo the maximum increase in the nonreducing sugars in the leaves during the day amounted to 1.54, 2.13, and 1.96 gm. per square meter of leaf for the corn and 2.52, 2.01, and 2.75 gm. for the same leaf area in the case of the milo. In the same three experiments the maximum increase in the reducing sugars amounted to only 0.20, 0.37, and 0.18 gm. per square meter of leaf in the case of the corn and 0.12, 0.70, and 0.00 gm. for a like area of leaf in the case of the milo. In the experiment with milo on August 3, 1917, and with corn on July 3 and 4, 1919, the maximum increase in the reducing sugars in the leaves during the day was respec-

TABLE IX.—*Daily variation of water, dry matter, and carbohydrates in the leaves of Dwarf milo and Red Amber sorgo at Manhattan, Kans., July 17 and 18, 1919*

PERCENTAGE OF THE CONSTITUENTS

Period ending—	Dry matter.		Water.		Total sugars.		Nonreducing sugars.		Reducing sugars.	
	Red Amber sorgo.	Dwarf milo.	Red Amber sorgo.	Dwarf milo.	Red Amber sorgo	Dwarf milo.	Red Amber sorgo.	Dwarf milo.	Red Amber sorgo.	Dwarf milo.
July 17.										
7 a. m.	22.4	21.4	77.6	78.6	1.74	2.28	0.83	0.76	0.82	1.41
9 a. m.	24.2	23.3	75.8	76.7	2.53	3.16	.88	1.41	1.53	1.61
11 a. m.	24.6	25.0	75.4	75.0	3.34	4.20	1.98	2.18	1.18	1.84
1 p. m.	26.0	26.0	74.0	74.0	4.06	5.17	1.85	2.82	2.02	2.01
3 p. m.	26.4	26.7	73.6	73.3	4.11	5.25	2.43	2.50	1.48	2.53
5 p. m.	25.6	25.9	74.4	74.1	2.17	4.88	1.38	2.36	.68	2.30
7 p. m.	25.3	25.5	74.7	74.5	2.81	5.49	1.97	3.30	.70	1.94
9 p. m.	24.9	24.0	75.1	76.0	2.33	3.57	1.25	2.06	.97	1.34
11 p. m.	25.0	24.5	75.0	75.5	2.13	2.13	.82	.78	1.21	1.25
July 18.										
1 a. m.	24.4	23.5	75.6	76.5	1.77	2.05	.28	.72	1.41	1.22
3 a. m.	23.4	22.9	76.6	77.1	2.13	1.86	.79	.48	1.24	1.29
5 a. m.	22.2	21.6	77.8	78.4	2.14	1.38	.86	.16	1.16	1.15
7 a. m.	21.0	20.5	79.0	79.5	2.07	1.32	.60	.16	1.38	1.10

GRAMS OF CONSTITUENTS PER SQUARE METER OF LEAF

July 17.										
7 a. m.	41.1	40.6	143.0	150.0	0.71	0.93	0.34	0.40	0.34	0.57
9 a. m.	42.4	41.2	133.5	136.0	1.07	1.30	.37	.58	.65	.66
11 a. m.	43.0	43.4	132.0	130.6	1.44	1.82	.85	.95	.51	.80
1 p. m.	45.2	44.6	128.7	127.1	1.83	2.30	.84	1.25	.91	.90
3 p. m.	46.5	46.6	129.8	128.4	1.91	2.45	1.13	1.16	.69	1.19
5 p. m.	45.7	45.5	133.3	130.6	.99	2.22	.63	1.07	.31	1.05
7 p. m.	45.4	45.3	134.3	132.4	1.27	2.49	.89	1.49	.32	.88
9 p. m.	44.4	43.1	134.6	139.9	1.03	1.54	.55	.89	.43	.58
11 p. m.	45.3	43.8	136.3	135.5	.96	.93	.37	.34	.55	.55
July 18.										
1 a. m.	44.4	43.0	138.2	140.4	.78	.88	.12	.31	.63	.52
3 a. m.	43.3	42.6	142.1	144.2	.92	.79	.34	.20	.54	.55
5 a. m.	42.3	41.3	148.9	150.1	.91	.57	.36	.07	.49	.47
7 a. m.	40.0	39.0	150.9	151.3	.83	.51	.24	.06	.55	.43

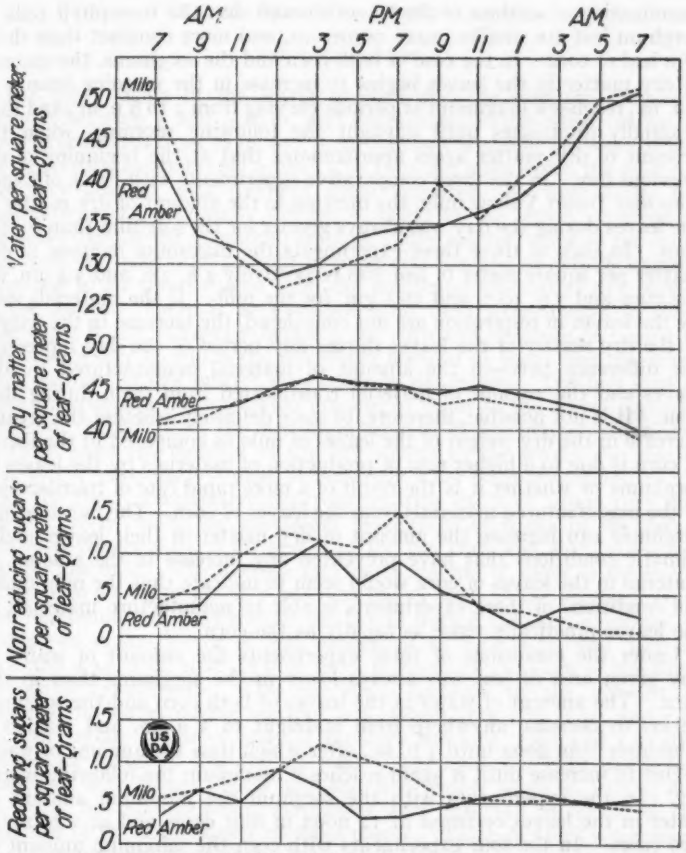

FIG. 6.—Graph showing the variation of the reducing and nonreducing sugars, dry matter, and water in the leaves of Dwarf Yellow milo and Red Amber sorgo during July 17 and 18, 1919.

The data in regard to the amount of water, dry matter, and carbohydrates obtained in each of the experiments are shown in Tables IV to IX. The results are expressed in percentages and in terms of the number of grams of constituents per square meter of leaf. The percentages of water and dry matter are calculated on a wet basis, while the percentages of carbohydrates are estimated on the amount of dry matter in the leaf. The changes in the amount of the constituents per square meter of leaf during each two-hour period of the experiments are shown by graphs in figures 1 to 6.

DRY MATTER AND WATER IN THE LEAVES

The daily variation of the water and dry matter in the leaves of corn and the sorghums has been reported in detail in a previous paper (*10*), so that only those facts will be mentioned that are essential to discussion of the variation of the carbohydrates of the leaves.

In the experiments the amount of dry matter in a given area of leaf was always greater for the sorghums than for the corn, and a microscopic

examination of sections of the leaves showed that the mesophyll cells of sorghum leaf are smaller, more numerous, and more compact than those of a leaf of corn. In the case of both corn and the sorghums, the amount of dry matter in the leaves begins to increase in the morning from 4 to 6 a. m., reaches a maximum at periods varying from 2 to 6 p. m., and then gradually diminishes until daylight the following morning, when the amount of dry matter again approximates that at the beginning of the previous day. In the three comparative experiments with Pride of Saline corn and Dwarf Yellow milo, the increase in the amount of dry matter in the leaves during the day was always greater for the sorghum than for the corn. In each of these three experiments the maximum increase in dry matter per square meter of leaf was respectively 4.8, 2.6, and 5.4 gm. for the corn and 7.6, 10.7, and 10.1 gm. for the milo. If the materials used by the leaves in respiration are not considered, the increase in the weight of the dry matter of the leaves during any period of the day represents the difference between the amount of material manufactured by the leaves and the amount of material translocated from them during that time. It is not possible, therefore, to state definitely whether the greater increase in the dry weight of the leaves of milo as compared to the leaves of corn is due to a higher rate of production of materials by the leaves of sorghums or whether it is the result of a more rapid rate of translocation of the manufactured materials from the leaves of corn. The fact that the sorghums can increase the amount of dry matter in their leaves under climatic conditions that have prevented any increase in the amount of material in the leaves of corn would seem to indicate that the milo under the conditions of these experiments is able to manufacture materials in the leaves practically twice as rapidly as the corn.

Under the conditions of these experiments the amount of water in any given area of leaf was always lower in the sorghums than in the corn. The amount of water in the leaves of both corn and the sorghum begins to decrease anywhere from midnight to 5 a. m. and reaches a minimum from noon until 3 p. m., after which time the amount of water begins to increase until it again reaches a maximum the following morning. In the experiments with the sorghum the minimum amount of water in the leaves occurred at 12 noon in four cases and at 1 p. m. in two cases. In the four experiments with corn the minimum amount of water in the leaves occurred three times at 2 p. m. and once at 3 p. m. In only one case did the quantity of water in the leaves appear to be the limiting factor in the production of dry matter. In the experiment on August 1, 1916, the amount of dry matter in the leaves of corn reached a maximum at 10 a. m. and remained constant until after 4 p. m., when it began to decrease. The amount of water in the leaves at 10 a. m. was 112.4 gm. per square meter of leaf, and it continued to decrease until it amounted to only 104.2 gm. per square meter of leaf at 2 p. m. In none of the other experiments with corn did the amount of water in the leaves reach a minimum lower than 117 gm. per square meter of leaf. In all of the other experiments the amount of dry matter in the leaves of corn continued steadily to increase past the time of the minimum water content of the leaves, so that it would appear that the minimum amount of water that can be reached in the leaves of corn and not interfere with the process of photosynthesis is slightly above 112 gm. per square meter of leaf. The minimum amount of water observed in the leaves of Dwarf Yellow milo and Red Amber sorgo in these experiments was 98 gm. per square meter of leaf, but this minimum apparently in no way retarded the production of dry matter.

THE CARBOHYDRATES OF THE LEAVES

THE TOTAL SUGARS

The total sugars in the leaves of corn and the sorghums begin to increase between 4 and 6 a. m. and reach a maximum which varied in these experiments from 12 m. to 5 p. m. After the maximum was reached the sugar disappeared rapidly until 9 to 10 p. m., after which the decrease was very gradual until daylight again appeared. In five of the ten observations the maximum amount of sugar in the leaves occurred at the same time as the maximum amount of dry matter, while in the other five cases the maximum amount of sugar was reached at periods varying from one to six hours earlier than the maximum amount of dry matter in the leaves. There is no evidence from these experiments to show that there is any difference between the corn and sorghum in regard to the time of day at which the maximum amount of sugar and the maximum amount of dry matter may occur in the leaves. The fact that in some experiments the maximum amount of sugar in the leaves occurred earlier in the day than the maximum amount of dry matter shows that in these cases the increase in the dry weight of the leaves was due in part to the temporary storage of materials other than the sugars.

In the three comparative experiments with Pride of Saline corn and Dwarf Yellow milo, the maximum percentage of sugar in the leaves was respectively 6.03, 6.33, and 6.44 for the corn and 8.27, 6.68, and 7.49 for the milo. The average maximum amount of sugar in the leaves of the milo was thus about 1 per cent higher than in the leaves of corn. The maximum sugar content of the leaves during the day, expressed in grams per square meter of leaf, amounted to 2.94, 3.25, and 3.37 for corn and 4.34, 4.11, and 3.83 for milo, respectively, in each of the three successive experiments. The maximum increase in the amount of sugar in the leaves during the day was respectively 1.73, 2.50, and 1.86 gm. per square meter of leaf for the corn and 2.63, 2.90, and 1.92 gm. per square meter of leaf for the milo. A comparative experiment was conducted with Dwarf Yellow milo and Red Amber sorgo on July 17 and 18, 1919, but no significant differences were observed in regard to the behavior of the total sugars in these plants. Table X gives a summary of the more important facts concerning the changes in sugars of the leaves during the day.

THE INSOLUBLE CARBOHYDRATES

In the experiment of July 25 and 26, 1917, the maximum amount of insoluble carbohydrates per square meter of leaf was reached at 6 p. m. in both the corn and milo, while the maximum sugar content occurred at 2 p. m. In the experiment with Dwarf milo on August 3, 1917, the maximum amount of sugar per square meter of leaf was reached at 12 m., but the maximum amount of insoluble carbohydrates did not occur until 8 p. m. In the observation on corn during the experiment of July 3 and 4, 1919, the maximum amount of sugar and insoluble carbohydrates per square meter of leaf was reached at 5 p. m. After the insoluble carbohydrates reached a maximum in the leaves, they showed little or no diminution in amount until about midnight, when they decreased rapidly until daylight.

TABLE X.—*Summary of the more important facts concerning the changes in the sugar in the leaves of corn and the sorghums during the day*

Date.	Plants.	Maximum amount of total sugars.		Maximum increase in the amount of—							
				Total sugars.		Nonreducing sugars.		Reducing sugars.		Dry matter.	
1916.		*Per cent.*	*Gm.*[2] [a]	*Per cent.*	*Gm.*[2]	*Per cent.*	*Gm.*[2]	*Per cent.*	*Gm.*[2]	*Per cent.*	*Gm.*[2]
July 20–21	Corn, Pride of Saline	6.03	2.94	3.28	1.73	2.96	1.54	0.36	0.20	3.1	4.8
	Sorghum, Dwarf Yellow milo	8.27	4.34	4.59	2.63	4.54	2.52	.21	.12	4.0	7.6
Aug. 1–2	Corn, Pride of Saline	6.33	3.25	4.81	2.50	4.09	2.13	.72	.37	4.0	2.6
	Sorghum, Dwarf Yellow milo	6.68	4.11	4.30	2.90	3.20	2.01	1.05	.70	5.5	10.7
1917.											
July 25–26	Corn, Pride of Saline	6.46	3.37	3.28	1.86	3.56	1.96	.22	.18	4.1	5.4
	Sorghum, Dwarf Yellow milo	7.49	3.83	3.28	1.92	5.28	2.75	None.	None.	5.1	10.1
Aug. 3	Sorghum, Dwarf Yellow milo	6.77	4.01	4.52	2.82	3.50	2.17	1.57	.87	5.1	9.6
1919.											
July 3–4	Corn, Pride of Saline	5.92	2.69	5.21	2.41	3.98	1.83	1.26	.60	3.9	6.2
July 17–18	Sorghum, Dwarf Yellow milo	5.49	2.49	3.21	1.56	2.54	1.09	1.02	.62	5.3	6.0
	Sorghum, Red Amber	5.06	1.91	2.37	1.20	1.60	.79	1.10	.57	4.0	5.4

[a] The abbreviation Gm.[2] is used to express the number of grams of the constituents per square meter of leaf.

The maximum increase in the dry matter of the leaves of corn and milo was always greater than the increase of the carbohydrates as determined by the methods used in these experiments. The case of Dwarf Yellow milo in the experiment of July 25, 1917, will serve as a concrete example of the above statement. On that date at 6 a. m. the dry matter in a square meter of leaf amounted to 45.3 gm. This increased gradually during the day until a maximum was reached at 6 p. m., when the dry matter amounted to 55.4 gm. per square meter of leaf. Thus each square meter of leaf increased in dry weight 10.1 gm. during the 12-hour period. The increase in sugars and insoluble carbohydrates from 6 a. m. to 6 p. m. amounted to 0.27 and 4.88 gm. per square meter of leaf, respectively, so that the total increase in dry weight in each square meter of leaf due to the carbohydrates amounted to 5.15 gm. There was thus an increase of 4.95 gm. of dry matter in each square meter of leaf that could not be accounted for by the increase of carbohydrates. In the several experiments the increase of dry matter during the day that could not be accounted for by the increase in sugar and insoluble carbohydrates during the same period varied from 7.4 to 53.7 per cent of the total increase of dry matter. Detailed information on this point can be obtained by consulting Tables VI, VII, and VIII. The undetermined material is evidently not protein, since uupublished data show that in the cases in question there is no increase in the absolute amount of nitrogen in the leaves during the day. The method used in obtaining the dry weight of a unit of area of leaf does not seem to be at fault, since the dry weight of a unit of area in all the experiments was approximately the same at the close of a 24-hour period as it was at the beginning. Since the samples used in determining the weight of a unit of leaf area at the beginning and at the close of an experiment were selected from rather widely separated portions of the leaf, it would seem that the structure of the leaf was uniform and that no appreciable error would be due to variations in the leaf samples that were used to determine the absolute amount of dry matter. The discrepancy observed between the total increase in dry matter in the leaves during the day and the increase in carbohydrates is due apparently to the fact that certain temporary storage products in the leaves are not detected by the methods used in determining the carbohydrates.

THE REDUCING AND NONREDUCING SUGARS

The nonreducing sugars in the leaves of the plants studied were, with the exception of the experiment with Dwarf milo and Red Amber sorgo, always in excess of the reducing sugars. The increase in the amount of the nonreducing sugars and the maximum point of increase of the total sugars is also the maximum point of increase of the nonreducing sugars. In the three comparative experiments with Pride of Saline corn and Dwarf Yellow milo the maximum increase in the nonreducing sugars in the leaves during the day amounted to 1.54, 2.13, and 1.96 gm. per square meter of leaf for the corn and 2.52, 2.01, and 2.75 gm. for the same leaf area in the case of the milo. In the same three experiments the maximum increase in the reducing sugars amounted to only 0.20, 0.37, and 0.18 gm. per square meter of leaf in the case of the corn and 0.12, 0.70, and 0.00 gm. for a like area of leaf in the case of the milo. In the experiment with milo on August 3, 1917, and with corn on July 3 and 4, 1919, the maximum increase in the reducing sugars in the leaves during the day was respec-

tively one-half and one-third of the increase of the nonreducing sugars. The highest ratio of the reducing sugars to nonreducing was that observed in the comparative experiment with Dwarf Yellow milo and Red Amber sorgo on July 17 and 18, 1919, when the maximum increase in the nonreducing sugars during the day was 1.09, and 0.79 gm. per square meter of leaf, respectively, for the milo and sorgo, while the maximum increase in the reducing sugars for an equal leaf area amounted to 0.62 gm. for the former and 0.57 gm. for the latter.

By consulting figures 1 to 6 it will be seen that the graphs representing the changes in the reducing sugars in the leaves of corn and the sorghums during the day are very irregular and that the time of the maximum increase in the amount of the reducing sugars very seldom if ever coincides with the time of the maximum amount of the nonreducing sugars. No significant differences were observed between corn and the sorghums in regard to the relationship between the reducing and the nonreducing sugars in their leaves. The observations made in these experiments in regard to the behavior of the nonreducing and the reducing sugars in the leaves of corn and the sorghums during the day and night are in accord with those of Brown and Morris (*1*), Parkin (*11*) and Davis (*3, 4, 5*) who worked, respectively, with the leaves of the nasturtium, snowdrop, and the mangold and potato. Since the nonreducing sugars increased so markedly in the leaves during the day and then decreased during the night, while the reducing sugars remained almost constant, these investigators concluded that the nonreducing sugars were the first sugars formed in the leaves in photosynthesis. It would seem, however, that it can not be determined definitely from chemical analyses whether the marked increase in the amount of the nonreducing sugars in the leaves during the day is due to the fact that they are the primary sugars of photosynthesis or to the fact that they are formed from the more simple sugars and accumulate in the leaves during the day as temporary storage products.

SUMMARY

In order to determine the changes of the carbohydrates in the leaves of corn and the sorghums during the day, analyses were made of material collected at two-hour intervals from the leaves of Pride of Saline corn, Dwarf Yellow milo, and Red Amber sorgo grown under identical or similar field conditions during the summers of 1916, 1917, and 1919. Ten sets of plants were under observation and the more important data obtained were as follows:

(1) The amount of dry matter in a given area of leaf of the sorghums studied was always greater than a like area of leaf of the corn. The dry matter in the leaves of both plants began to increase at daylight, reached a maximum at periods varying from 2 to 6 p. m., and then gradually diminished until daylight the following morning. In the comparative experiments with corn and milo the maximum increase in dry matter per square meter of leaf during the day was approximately twice as great in the leaves of milo as in the leaves of corn.

(2) The amount of water in a unit of leaf area was always greater in the leaves of corn than in the leaves of the sorghums. The water content of the leaves of both plants began to decrease anywhere from midnight to 5 a. m., reached a minimum from 12 m. until 3 p. m., and then began to increase until a maximum was reached the following morning.

In the case of corn the amount of water seemed to become a limiting factor in the production of dry matter when it reached a minimum of about 112 gm. per square meter of leaf. The water content of the sorghum leaves reached a minimum of 98 gm. per square meter of leaf, but this minimum apparently in no way retarded the production of dry matter.

(3) The total sugars in the leaves of the plants under observation began to increase between 4 and 6 a. m., reached a maximum which varied from 12 m. to 5 p. m., and decreased gradually from that time until daylight the following morning. In the 10 observations the maximum amount of sugar in the leaves occurred in five cases at the same time as the maximum amount of dry matter, while in the other five cases the maximum sugar content was reached at periods varying from one to six hours earlier than the maximum amount of dry matter. There is no evidence to show that there is any difference between corn and the sorghums in regard to the time of day at which the maximum amount of sugar and dry matter may occur in the leaves.

(4) The insoluble carbohydrates were estimated as starch according to the official method of acid hydrolysis and thus include besides starch the pentosans and other insoluble carbohydrates that are converted into reducing sugars by boiling with hydrochloric acid. The insoluble carbohydrates thus estimated generally reached a maximum later in the day than the sugars and after they had reached a maximum showed little decrease until about midnight, after which they decreased rapidly until daylight. The total increase in the dry matter of the leaves during the day could not be accounted for by the increase in the sugars and insoluble carbohydrates during the same period. The increase in the total sugars and insoluble carbohydrates in the leaves during the day only approximated from 46 to 92 per cent of the total increase in the dry matter of the leaves for the same period.

(5) The nonreducing sugars in the leaves of the plants studied were, with the exception of the experiment with Dwarf milo and Red Amber sorgo, always in excess of the reducing sugars. The nonreducing sugars increased markedly during the day and decreased during the night, while the reducing sugars, as a rule, showed very little increase, and the amount present at the different periods of the day was very irregular. The maximum increase in the reducing sugars in the leaves during the day in the case of corn and milo only amounted to from one-tenth to one-third the increase in the nonreducing sugars. No significant differences were observed between corn and the sorghums in regard to the relationship between the reducing and nonreducing sugars in their leaves.

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